READING BETWEEN THE LINES: INDICATORS OF DEVELOPMENTAL STRESS IN PREHISTORIC OHIO VALLEY CHILDREN FROM LINEAR ENAMEL HYPOPLASIAS

by

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A thesis

submitted in partial fulfillment

of the requirements for the degree of

Master of Arts in Anthropology

Boise State University

May 2016

BOISE STATE UNIVERSITY GRADUATE COLLEGE

DEFENSE COMMITTEE AND FINAL READING APPROVALS

of the thesis submitted by

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Thesis Title: Reading Between the Lines: Indicators of Developmental Stress in Prehistoric Ohio Valley Children from Linear Enamel Hypoplasias

Date of Final Oral Examination: 01 March 2016

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DEDICATION

For my grandfather. I wish you were here to celebrate this achievement with me.

ACKNOWLEDGEMENTS

First, I would like to express my gratitude to my advisor, Dr. Samantha Blatt. This thesis would never have been possible without her guidance, expertise, enthusiasm, and (especially) her samples. I cannot thank her enough for entrusting to me the very casts and photomontages that she created for her dissertation. I would also like to thank my other committee members, Dr. Mark Plew, and Dr. Pei-Lin Yu, for their contributions, comments, and advice in the development of not only this thesis, but on other aspects of my academic career.

I would like to thank Dr. Kristin Snopkowski for her collaboration on the statistical methods used to evaluate my research questions, and to Jordan Wohlert for her help in displaying the results of some of those statistical tests.

I am also very grateful to the American Indian Advisory Committee of Dayton, Ohio who permitted the original nondestructive and destructive techniques of the teeth from the SunWatch Indian Village. This thesis could have never existed without the merit of the histologically-derived data that I used. It is my hope that this project perpetuated their efforts enhancing the discovery of the past lifeways of their ancestors. Additionally, the dissertation from which my data is based was possible due to funding through multiple sources, including the Wenner-Gren Foundation for Anthropological Research, the Larsen Grant, Sigma Xi Grants-in-Aid-of Research via the Ohio State University Chapter, and the alumni Grant for Graduate Research and Scholarship.

Further, this thesis was also funded through the Dan Montgomery Research Award, and Sigma Xi Grants-in-Aid-of Research.

To my dear friends, Mallory Schreier and Kathy Petersen, for their companionship, their feedback, and their continued perseverance with me through these last two years of our shared graduate experience. To James Shawver, who was always (literally) behind me every step of the way to the completion of this project. And to Graham, my constant stress relief, who never failed to make me smile, even during the cloudiest of days.

Lastly, to my mother and my sister, whose love, inspiration, and unwavering support has encouraged me in every endeavor, despite my own confidence.

ABSTRACT

A large body of research in bioarchaeology focuses on the changes in the human skeleton associated with the introduction of agriculture. It is assumed that the intensification of agriculture results in an increase in physiological stress and poor health. However, previous studies have shown that stress experiences cannot be generalized by subsistence strategy and prevalence comparisons alone. Rather, age-at and duration of stress events are necessary to construct patterns of health.

Linear enamel hypoplasias (LEH) of subadult permanent dentition serve as a proxy for understanding health and stress in archaeological populations. LEH are defects in enamel, characterized by an increased distance between enamel growth rings, known as perikymata, across the tooth. Since enamel does not remodel, LEH provides a more accurate record of developmental disruption than other skeletal indicators. By analyzing LEH, this project aims to investigate the time and duration of stress events of foragers compared with those of agriculturalists.

The sample is made up of the adult anterior teeth of 40 children of a foraging group (Duff) and two agricultural populations (SunWatch and Buffalo) from the prehistoric Ohio Valley. The ages-at-death of these children have been previously determined using highly accurate dental cellular histology, capable of estimating age to the day. The first step in this project was the microscopic imaging of the surfaces of epoxy cast replicas of the subadult dentition. A scanning electron microscope was used to create 50X photomontages of micrograph images of the replicated tooth surfaces. From

these photomontages, LEH from systemic stress was recorded as those that concurrently match among other teeth from the same individual. Individuals with observable defects on two or more teeth were categorized as LEH positive. To calculate the ages at which stress events occurred, perikymata were counted from the occlusal surface of each tooth to the beginning of each identified LEH, then added to cuspal enamel formation times, and initiation times for each tooth. The total number of perikymata within each defect furrow were used to reflect the duration of the stress events. These variables were compared between samples using Fisher's exact tests, boxplots, pairwise ANOVA tests, and averages.

The results provide a comparative analysis of the changes and continuities in the lifestyles of foragers and agriculturalists in the Ohio Valley. Because their environments were not markedly different, variation in stress events observed between these groups are the result of cultural or nutritional change evidence of which contributes to the evaluation of political or migratory interaction between regional groups. Indeed, it appears that SunWatch children endured the most stress events, although Duff children suffered the longest stress events. Age-at-death among the SunWatch sample was the youngest, therefore their higher frequency of LEH occurrence suggests a decline in quality of health compared to earlier foraging populations. Results are similar to previous studies exploring transitions in health with the rise of agriculture.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: INTRODUCTION

The Problem

One of the most significant areas of research in bioarchaeology focuses on changes in the human skeleton associated with the introduction of agriculture because the transition is often viewed as the beginning of a series of changes in social organization (Stock and Pinhasi. 2010). These changes develop on the basis of the rise of food production and the storage of food surplus, leading to, for example, property ownership, social hierarchy, specialization, and technological evolution (Hayden, 1990; Milner, 2004). These economic and cultural changes are influential determinants of the patterns of morbidity, mortality, and stress (Cassel, 1976; Dubos, 1965; Hinkle, 1974). Here, stress is used as a general term to describe physiological disruption from homeostasis, or rather for any indication of decreased ability to adapt biologically to the environment (Goodman et al., 1984a).

Under circumstances conducive to increased stress, such as poor nutrition, population aggregation, and increased infectious disease, skeletons and dentitions exhibit stress indicators in elevated prevalence (e.g. Goodman and Armelagos, 1985; Guatelli-Steinberg et al., 2004; Hillson, 1992a; Hutchinson and Larsen, 1988; King et al., 2005). Indeed, the intensification of agriculture is heavily correlated with an increase in skeletal indicators of physiological stress relative to hunter-gatherers in similar environments (Cohen and Armelagos, 1984; Cohen, 1989; Steckel and Rose, 2002; Larsen, 2006). The impact of the adoption of agriculture on the stress experience in earlier human populations is affirmed in a number of settings when changes in health and nutrition are documented by archaeological and osteological means. 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). Having said that, this trend is not universal. In a comparison of late prehistoric and contact-era maize agriculturalists from one area of the American Southeast, there is a decrease in stress prevalence (Hutchinson and Larsen, 1988). However, the stress episodes were of either longer duration, or greater severity, or both. This trend is characteristic in other parts of the world, in which initial stressors are alleviated as technology improves or as agricultural populations expand into new habitats (see Winterhalder and Kennett, 2006).

With that in mind, generalizations about the health of archaeological populations cannot be made solely based on subsistence strategy or stress experience. Instead, it is necessary to consider the duration of each stress episode and the ages at which they occurred (Temple et al., 2013). Such an analysis is possible through the examination of dental growth disruptions caused by physiological stress. More specifically, disruption in dental enamel is commonly studied as a stress indicator as it provides an invaluable source of information on past morbidity and health because it grows in regular, recognizable patterns and the effects of disruptions to growth are well documented, and will be discussed in Chapter 3. Since teeth do not remodel throughout an individual’s lifetime, markers of stress indicate growth disruptions during childhood, while teeth are growing (Hillson, 1992a).

Although outside the scope of this project, there are a wide variety of stressors that are expected to impact children’s health and nutrition among agricultural populations which differ from those among foragers. These include close birth spacing (Armelagos et al., 1991), early weaning (Franklin et al., 2010; Katzenberg et al., 1996), and low quality weaning foods (Wright, 1997), inter-sibling competition (Sulloway, 2007), labor demands, and societal conflict (Boone, 1992; Walker 2001). Within ranked agricultural societies, these stressors are not expected to affect all children in the same manner, where lower ranked individuals experience increased physiological stress (Sapolsky, 2005).

Indeed, children provide an abundance of information through their skeletal and dental remains regarding their physical and social lives and deaths, including the cultural approaches of their society toward children. Bioarchaeological inquiry into the diversity of mortuary treatments of children, weaning, diet, and social and labor roles is made possible through the investigation of ages and health of children from the archaeological record. What is more, understanding health and stress in archaeological populations stems from the analysis of childhood growth disruption since children are the most susceptible to environmental insults (Blatt, 2013). In turn, these periods of susceptibility are a major factor in the outcomes of human skeletal variation in adults, which is a hallmark of biological anthropology. Understanding and recording markers of human-environmental interaction is significant to the holistic view of human growth and development through time and space.

Purpose

This project aims to reconstruct patterns of stress via growth disruption from subadult teeth using linear enamel hypoplasia (hereafter LEH) prevalence, age of occurrence, and duration among temporally distinct populations with different subsistence strategies (foraging and agricultural) and social structures within the prehistoric Ohio Valley. The objectives of this project are to investigate the following from the target populations:

1. the number of stress episodes (LEH) each individual experienced;
2. the ages at which stress events (LEH) occur;
3. the duration of these stress events (LEH);
4. the timing and duration of stress events (LEH) among populations.

The central hypothesis being explored is if no differences in LEH frequency and duration will be significantly different between the hunter-gatherer and agricultural populations of the Ohio Valley. Following previous research (e.g. Cohen and Armelagos, 1984; Larsen, 2006), it is predicted that the agricultural populations will show a statistically significant increase in physiological stress by exhibiting a higher frequency and longer duration of LEH than the foraging population. Regarding the age of occurrence during which stress incidents occur, (e.g. Corruccini et al., 1985; Goodman et al., 1980; Hillson, 1979; Hutchinson and Larsen, 1995), it is predicted that a significantly greater frequency of LEH will occur between the ages of two and four years due to the the sensitivity of children under the age of five years to environmental and cultural insults (Goodman and Armelagos, 1989).

Although it is not currently possible to specify the prehistoric stress events that the LEH represent, this project will present a biocultural view of growth disruption using archaeological evidence. The foreign political and social influences (not to mention evidence of genetic flow and drift) from the Mississippian culture in the Fort Ancient Late Prehistoric culture of the Ohio Valley are of particular temporal significance to this project and have been of recent interest to Eastern Woodland archaeologists (i.e. Cook, 2004; 2008; Sciulli, 1990; Sciulli and Oberly, 2002). In light of the archaeological record, biological evidence from this thesis is therefore an important contribution to assessing the political and migratory interactions in the past by comparing stress experiences between populations with varying degrees of external influence from other Eastern Woodland groups.

Chapter Organization

This thesis consists of seven chapters, including this introduction which has presented the purpose, problem, and hypotheses. Chapter 2 presents the cultural background of the samples used in this investigation with specific discussions of the relevant archaeological and bioarchaeological findings, including environment, diet, and health. These may play a role in any variations seen in the stress experiences between the target populations (SunWatch, Buffalo, and Duff). Chapter 2 also includes a review of skeletal and dental indicators of stress and addresses temporal and regional trends in the health of prehistoric Ohio Valley groups. Chapter 3 introduces the structures of macroscopic dental anatomy, and enamel microstructures necessary to understand the biological basis behind the methods and theory applied by this study. Chapter 3 also reviews the literature on linear enamel hypoplasias (LEH) to underscore their utilization when reconstructing the health of archaeological populations. Chapter 4 discusses the materials and methodology employed in this study. Chapter 5 compiles the results of this study in quantitative form, followed by a comparative discussion by placing the results in a broader context in Chapter 6, especially as they relate to the cultural-historical background of each sample. Chapter 7 reviews this project, considering additional possibilities for deviations in the results, concluding with avenues of future investigation.

CHAPTER 2: CULTURAL BACKGROUND AND SITE DESCRIPTIONS

Introduction

This chapter summarizes and describes the archaeological and biological background of the target populations. This is necessary to provide a clear cultural, historical, and environmental context to the hypotheses tested in this study. First, an overview of settlement and subsistence characteristics of the Archaic, Woodland, and Late Prehistoric periods is presented. Subsequently, from their broader temporal/cultural context, the sites from which data were collected are described. Namely, the Duff site (22LO111), representing a terminal Late Archaic cemetery, and the SunWatch (33MY57) and the Buffalo (46PU31) sites as representative of Fort Ancient/Late Prehistoric villages are introduced. Finally, temporal patterns of health from skeletal indicators of stress are addressed with a focus on trends in prehistoric Ohio Valley populations.

Cultural-Historical Background

The region encompassing the Upper and Lower Ohio Valley consists of Ohio, Pennsylvania, West Virginia, and Kentucky. The majority of the valley is comprised of the hilly, unglaciated Appalachian plateau whose soils have a low calcium content and low to moderate fertility (Sciulli and Oberly, 2002). The ecologically diverse till plain to the west of the plateau extends through central and western Ohio. The high-calcium soils of this region supported more open areas of forest with pockets of prairie vegetation. Ohio is divided into the glaciated plains and hills of the northwest and the unglaciated Appalachian plateau of the southeast. The till plains, lake shores, and large alluvial valley bottoms between this geological rift equip this region with diverse ecosystems. This great resource potential was the focus of prehistoric population concentration.

The culture-historical record of native populations of the Eastern Woodlands is divided into five phases: Paleo-Indian (12000-6000 BP), Archaic (6000-3000 BP), Woodland (3000-1100 BP), Late Prehistoric (1100-400 BP), and Proto-historic (400-200 BP) (Fagan, 2005). Biocultural studies are possible only for populations since the latter part of the Archaic period because it is not until this time that human remains were disposed of in large cemeteries (Griffin, 1983; Sciulli and Oberly, 2002).

Overview of Archaic Period

Throughout the Archaic phase, human populations appear to have been growing and expanding into regions that were previously underutilized (Brose, 1979). Populations lived in small (though relatively larger compared to the Paleo-Indian period), scattered groups at seasonal habitation sites subsisting on a mixture of hunting and gathering, with a focus on terrestrial game, nut harvesting, and riverine resources (Parmalee, 1969). There is evidence of collection and early domestication of seeds, squash, and gourds (Eastern Agricultural Complex domesticates), which play an increasingly important role in subsistence from Middle Archaic through Woodland periods (Smith, 1989).

The Late Archaic phase (ca. 3000 BP) witnessed an intensification in occupation as evidenced by an increase in both population and site size. Additionally, a number of regionally distinctive burial practices developed that intensified cultural differentiation. The area between the Ohio River and the Great Lakes manifested circumscribed mortuary complexes, such as “Glacial Kame”, in northwestern Ohio and into Indiana, Michigan, and Ontario, as well as “Red Ocher” in south-central Ohio (Sciulli and Oberly, 2002).

Non-local burials goods also held an increasing importance during this time (Lepper, 2005; Milner, 2004).

Overview of Woodland Period

Populations of the Early Woodland phase (ca. 3000-2000 BP) exhibit strong biocultural similarities with Late Archaic populations. The distinctive features of the Early Woodland populations are merely elaborations of Late Archaic features, developing into an emphasis on burial mound building (Sciulli and Oberly, 2002). During this time, foraging subsistence is still employed, with the presence of Eastern Agricultural Complex domesticates (Smith, 1989). Early Woodland populations still occupied small multiple seasonal sites, but began to manage small gardens with domesticated plants.

Transitionally, the settlements in the Middle Woodland (2100-1500 BP) were patterned in a dispersed system of hamlets with central ritual districts characterized by mounds and earthworks (Dancey, 1994). By this time, most of the Eastern United States was covered by a Hopewellian Interaction Sphere, a vast trade network of local and interregional exotic materials. Elaborate burial mounds of important individuals are spread throughout the southeast and north of the Ohio River. The region from Illinois to Ohio contains the most archaeologically visible area of burial ceremonialism from this period which is known as the Hopewell tradition. Researchers assume a common body of religious practice and cultural interaction due to the similarity of earthworks and burial goods (Church, 2002; Lepper, 2005; Milner, 2004).

By the Late Woodland phase (1500-1100 BP), many populations within the middle and upper Ohio Valley witnessed some fundamental changes to the settlement patterns, material culture, and ritual activities than previous phases (Church and Nass, 2002; Sciulli and Oberly, 2002). Groups resided in larger and more permanent settlements located on bluffs of major streams or rivers. Some were fortified with ditches or embankments and others were organized around central plazas. The construction of corporate burial mounds decreased drastically in most areas, as well as the long-distance trade in exotic materials (Lepper, 2005). Subsistence during this time was similar to that during the Middle Woodland period, although the beginnings of cultivation greatly supplemented the Eastern Agricultural Complex domesticates.

Overview of Late Prehistoric Period

Beginning around 1100 BP, the Late Prehistoric period (ca. 1100-400 BP) is marked by changes in subsistence and population structure. Maize appeared consistently in the record and it became the most important element in the food economy as it allowed seasonal control of food yields and storage (Cook, 1984). These changes are accompanied by changes in community size, social complexity, regional integration, and extra-regional trade. Associated with this subsistence shift is the presence of relatively large villages located in major river valleys (Milner, 2004).

Though settlement structure varied, they were generally nucleated, larger, and more permanent than during the Late Woodland (Lepper, 2005). Many of these settlements were enclosed by palisades or built on defensive bluffs overlooking the landscape. Unlike Late Woodland settlements, the social and ritual space became combined with plazas, perhaps indicating a greater need for public ritual in daily life (Seeman, 1992). This can also be interpreted as an increased interdependency around household units after the introduction of labor intensive subsistence strategies. While large cemeteries are usually associated with these villages, mortuary ceremonialism as seen in the Early and Middle Woodland periods is virtually absent (Sciulli and Oberly, 2002).

The Late Prehistoric period is characterized by five parallel, geographical, indigenous cultural traditions. The Fort Ancient culture centered in the south and southwestern areas, Sandusky in the northwest, Monongahela and Belmont in the east, and Whittlesey in the northeast (Pollack and Henderson, 2000; Sciulli and Oberly, 2002).

Terminal Late Archaic: The Duff Site

The Duff site, one of the three sites from which materials were drawn for this study, is classified as a terminal Late Archaic cemetery. As such, the following section will provide a summary of the terminal Late Archaic period (ca. 3000 B.P.) of the Eastern Woodlands to serve to give a general archaeological context of the site. Additionally, it will provide a means of comparison to the two Fort Ancient sites utilized in the sample.

Compared with small, dispersed Ohio Valley populations in the previous millennia, archaeological evidence has shown that the Late Archaic witnessed a number of fundamental biocultural changes (Brose, 1979; Griffin, 1983; Sciulli, 1990). Subsequently, these changes formed the basis of the socio-culturally more elaborate and complex Adena and Hopewell traditions of the Woodlands period. The discovery of Late Archaic sites in a wider range of regions compared to previous periods indicates an increase in population size and density. Additionally, many sites appear to have been repeatedly occupied (Brose, 1979: Griffin, 1983). While subsistence economy remains unchanged, there is considerable widespread evidence for specialization in settlement patterns, an elaboration of mortuary ceremonialism, and intensification of long-distance transportation of raw materials and manufactured goods (Griffin, 1983).

Subsistence Economy and Diet

The majority of evidence for the subsistence economy of Archaic peoples comes from the Middle - Late and terminal Late Archaic periods (ca. 4000 - 3000 B.P.) (Griffin 1952; Sciulli and Oberly, 2002). The initial phase of the Late Archaic period saw the establishment of modern ecological systems in the lower Great Lakes region (Ford, 1977). Inhabitants of the Ohio Valley during this time were opportunistic hunter-gatherers who lived in a mosaic of deciduous forest and prairie with diverse faunal assemblages such as deer, birds associated with terrestrial habitats, fish, and freshwater mussels (Parmalee, 1969).

Similar to previous periods, the archaeobotanical record shows that wood charcoal and nutshell continued to dominate assemblages. Seeds become increasingly common, suggesting the increasing importance of propagation of certain weedy plant species (Emerson et al., 2009). During the terminal Late Archaic, there is a shift away from a focus on nuts (Asch and Asch, 1985) and back to diversification, seemingly structured to meet the needs of increasingly sedentary groups (Asch and Asch, 1986). Domesticates, such as maize, were neither produced or consumed, a theory supported by isotopic evidence (Stothers and Bechtel, 1987).

Cemeteries and Mortuary Practices

Formal cemeteries first appear in the Middle Archaic and become widespread by the terminal Late Archaic across much of the Midwest (Buikstra and Charles, 1999; Spence, 1986; Stothers and Abe,l 1993). The development of cemeteries occurred once people settled into smaller territories and began using resources more intensely than their earlier predecessors (Brown, 1983; Brown and Vierra, 1983). Cemetery development also coincided with overall population growth around 4000 B.P. as evidenced by an increase in the number of sites across the Eastern Woodlands (Milner, 2004). The influence of burial location can be influenced by resource availability in a specific location, therefore it is necessary to analyze multiple sites for a more complete picture of mortuary practices (Charles and Buikstra, 1983).

Treatment of remains varied by region, but of particular importance to this inquiry, are the regionalized mortuary practices referred to as “Red Ochre” (found in south-central Ohio), or “Glacial Kame” (found in northwestern Ohio) (Sciulli and Oberly, 2002). These are characterized by red ochre in the graves. These burials are typically flexed in shallow, sandy pits whereas “Glacial Kame” burials are found in glacial grave knolls and are identified from the presence of a three-hole, sandal-sole gorget made from a marine shell as well as commonly found copper beads and awls and birdstones. These mortuary traditions show similarity in addition to geographic overlap suggesting a shared common ancestry or a participation in a wider diffusional network (Mason, 1981; Sciulli and Aument 1987).

Formal cemeteries also suggest that populations began to organize themselves in circumscribed areas in the terminal Late Archaic. In one study determining biological differentiation of eight terminal Late Archaic cemeteries, including the Duff site, Sciulli (1990) found an overall similarity in cranial metrics that strongly suggests that the samples represented populations that shared a recent common ancestor. The analysis of discrete trait variation in the same study showed that the biological distances between samples are significantly associated with the geographical distances. In other words, the geographically closer the populations, the more related they were.

Duff Site (22LO111)

The Duff site solely consists of a relatively large and complete cemetery. It was located 35 km southeast of Lima, Ohio, and 200 meters east of the Great Miami River. The cemetery, discovered and salvaged in 1984, appears to have been used for a short period of time (Sciulli and Aument, 1987). It is considered that all interred individuals were recovered and accounted for a large percentage of each skeleton. Most burial pits contained a single individual in a flexed position with hands covering or located near the face (Sciulli and Aument, 1987; Sciulli, 1990). Artifacts associated with the burials indicate that the Duff site is a typical terminal Late Archaic Glacial Kame burial complex, given the shell and copper beads, red ochre, and projectile points. However, sandal-sole-shaped gorgets are absent from the Duff site, which is a hallmark artifact of the Glacial Kame complex. Nevertheless, radiocarbon dates from skeletal material indicate a terminal Late Archaic site use (Sciulli and Aument, 1987; Sciulli, 1990).
A total of 102 individuals were recovered, including 58 adults, and 44 subadults, of which 90 could be aged and 49 sexed (Sciulli and Aument, 1987). A paleodemographic study of the Duff population by Sciulli and Aument (1987) using life table methodology reveal:

- approximate balances in sex ratio
- high frequency of death in the first year of life
- 8% survival rate after age 40
- high young adult mortality and low infant mortality
- correlation between an increase in dental pathology around age 20, with an increase in mortality in the same age cohort

- life expectancy at birth was 19.2 years
- crude death rate (0.052) was comparable to other populations
- overall patterns were similar to prehistoric, pre-contact populations

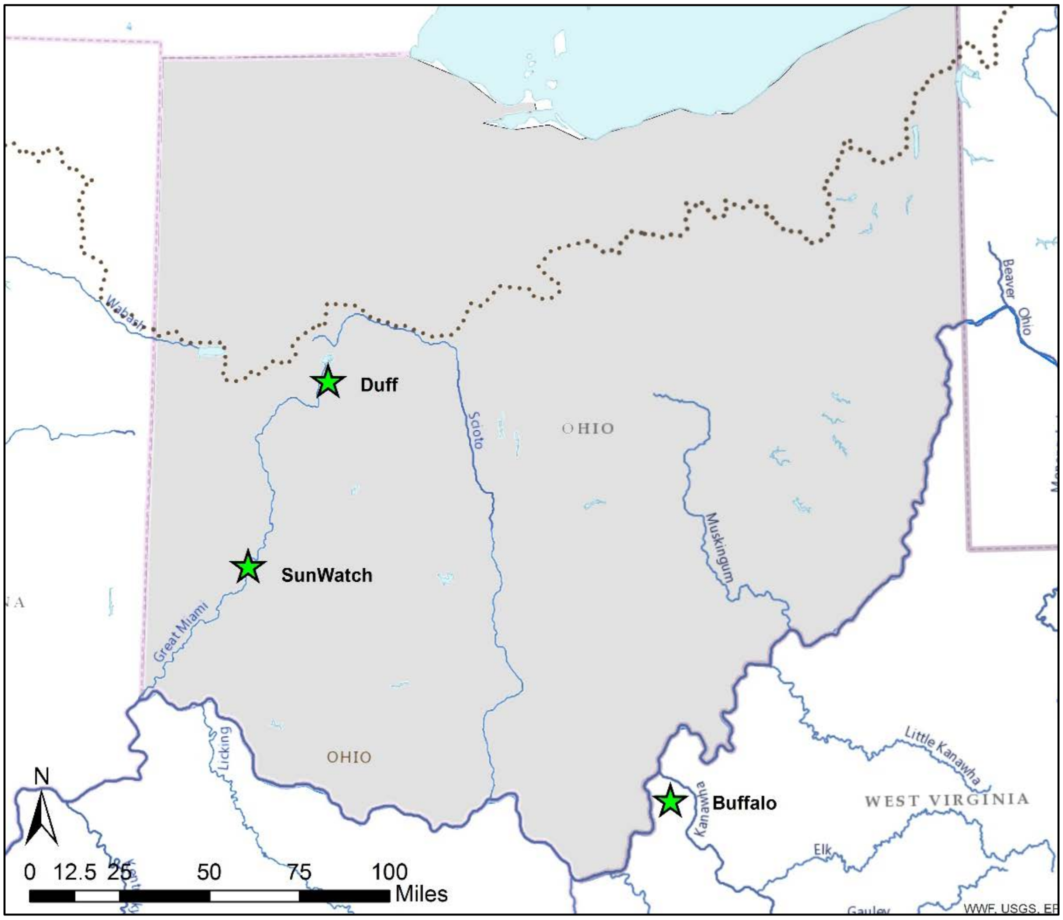

Figure 1: Map of Ohio showing the locations of the sites used in this study.

Fort Ancient Period: The SunWatch and Buffalo Sites

The Fort Ancient tradition is commonly referred to as the Late Prehistoric period, but the latter is a temporal period covering numerous other cultures contemporaneous to the former. Fort Ancient refers to sedentary village horticulturalists who lived in the middle Ohio Valley between A.D. 1000 and A.D. 1750. It included the southeastern edge of Indiana, the southern third of Ohio, the central and eastern portions of Kentucky, and the western West Virginia (Dunnell, 1971; Drooker, 1997; Essenpreis, 1978; Griffin, 1943). The region is conceived of being inhabited by tribal groups within an environmentally diverse area in which the Fort Ancient "culture" is not a homogenous unit (Sahlins, 1968; Henderson and Pollack, 2001). Instead, this "culture" is an arbitrary regional grouping of autonomous villages responding in kind to varied circumstances and varying degrees of Mississippian influence (Cook, 2007). There are four temporal units commonly used as a framework for describing, ordering, and comparing Fort Ancient cultural expressions within and between areas. They are: Early Fort Ancient (A.D. 1000-1200), Middle Fort Ancient (A.D. 1200-1400), Late Fort Ancient (A.D. 1400-1550), and Protohistoric (A.D. 1550-1750) (Griffin, 1943).

Early Fort Ancient is characterized by family hamlets with kinship as the prime social organization principle (Pollack and Henderson, 1992). Interregional variation in material culture is found in Middle Fort Ancient while region-wide similarities are found afterward. The transition from Middle to Late Fort Ancient is marked by an increase in village size and the appearance of their circular forms. Fort Ancient groups abandoned the Ohio Valley sometime during the Protohistoric period, although it is unclear if this is due to a process of decreasing population or decreasing archaeological visibility of settlement patterns (Henderson and Pollack, 2001).

One of the most significant features of the Fort Ancient period overall is the maize-intense diet that focused less on starchy-oily seed crops and nuts compared to earlier periods (Rossen, 1992). Stable isotope data ($^{13}C/^{12}C$) indicates that maize was by far the most consumed food by the Fort Ancient people, although some supplementary hunting and gathering was still employed to offset the risks of solely depending on maize.

While maize first appears in the archaeobotanical record in the Eastern Woodlands around 2000 B.P., it was not until the Late Woodland period (A.D. 400 - 1000) that its consumption increased in popularity (Buikstra, 1992; Riley, 1994). Many of the patterns of health problems, including the high prevalence of dental disease (Sciulli and Oberly, 2002), suffered by Fort Ancient groups may have arisen from chronic protein malnutrition related to a maize-intense diet. This is arguably an adaptive strategy as this dietary shift also permitted high fertility. Fort Ancient populations also tended to have shorter stature, a lower life expectancy, and high toddler mortality than earlier hunter gatherers (Cassidy, 1984).

SunWatch Site (33MY57)

The SunWatch village is the most extensively excavated and analyzed Fort Ancient site and serves as the type site for Middle Fort Ancient (A.D. 1200-1400) settlement form and social structure due in part to its excellent preservation, coherency, and relatively short-term occupation (Heilman et al., 1988; Henderson and Pollack, 2001). The site is located on the west bank of the Great Miami River, three miles south of Dayton, Ohio. SunWatch is thought to have seen one or two occupation periods between A.D. 1150 and 1450, as evidenced by radiocarbon dates and archaeological temporal indicators (Heilman et al., 1988; Cook, 2004). The skeletal collection consists of 63 adults and 103 subadults. It was curated and is housed by the Dayton Society of Natural History at the Boonshoft Museum of Discovery, Dayton, Ohio.

The site was formally excavated from 1968 until 1988, revealing a well-planned circular village, numerous burials, storage pit structures, hearths, and houses, organized around a red cedar center pole, and culminated in a stockade around the periphery

(Heilman and Hoefer, 1981). The site, as seen in Figure 2, displays clear spatial patterning indicative of an autonomous village with kin-centered households organized in dual corporate organizations (Cook 2004; 2012). The investigation of a pottery assemblage and selected burial features concluded that SunWatch was organized in a dual division structure with possible components consisting of localized households, clans, sodality, and elite/ritual area (Cook, 2004). Burials are clustered in kin groupings and are differentiated by age and sex (Evans-Eargle, 1998).

The overall subsistence strategy for SunWatch is described as maize-intensive horticulture with local seasonal supplementary foodstuffs (Shane, 1988; Wagner, 1988). Carbon isotope analyses have estimated that 48-75% of the diet was maize (Kennedy, 2000; Broida, 1983; Conrad, 1988). This proportion of maize consumption is similar to other Late Prehistoric and Protohistoric groups (Katzenberg et al., 1995; Stothers and Bechte,l 1987). The heavy reliance on maize is further supported by the presence of many deep storage pits indicating risk management since these pits would have acted as buffers against food shortages. Carbon isotope analysis also reveals there is no difference between males and females in maize consumption. However, according to nitrogen isotope analysis, females ate less meat than males. This is a common result in other studies, reflecting either gendered division of labor or status (Conrad, 1988).

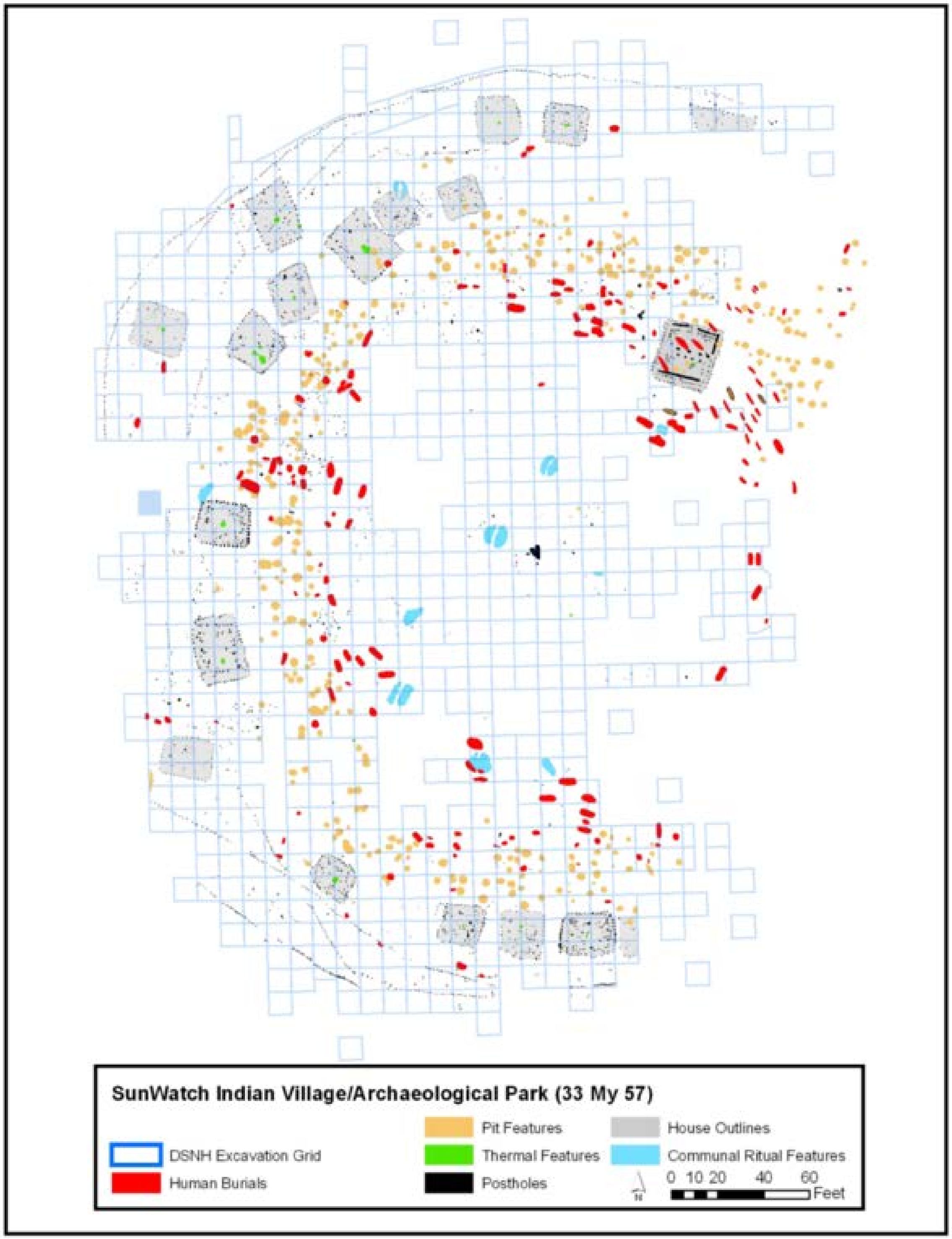

Figure 2: SunWatch Village and archaeological features. From Blatt, 2013, modified from Gosman,, 2007.

Buffalo Site (46PU31)

The Buffalo site is located near the town of Buffalo on the east bank of the Kanawha River, 15 miles upstream from its junction with the Ohio River in West Virginia (Blatt, 2013). Formal excavations took place from 1963-1965 during which emphasis was placed on determining the settlement layout and house patterns. Topsoil was cleared by bulldozer to reveal underlying features. Of the approximately 500,000 square foot area, only 67,000 square feet were excavated (Hanson, 1975). This broad-scale effort resulted in poorly recorded burials and grave goods. Drooker's (2000) mortuary analysis reveals that during the excavation seasons, burials were avoided, crushed, disturbed, and displaced by the bulldozer. Therefore, the burials represent minimums. The skeletal sample that was collected is now curated by the Grave Creek Archaeological Complex, Moundsville, West Virginia (Blatt, 2013).

The site spans several temporal/cultural periods: Archaic, Middle Woodland, early Middle Fort Ancient, and Late Fort Ancient. Since the Buffalo sample used in this study dates to the Fort Ancient period, only these occupations will be discussed. The Fort Ancient occupation consists of two overlapping villages, referred to as the Downstream and Upstream villages (Hanson, 1975). Both villages were oval in plan, consisting of an outline of palisades (see Figure 3), with an inner ring of house structures, and near featureless central plazas. House structures, burials, and refuse areas were concentrically aligned between the palisades and plazas. While there is scant evidence supporting claims of kinship groups, house sizes were spacious enough to accommodate large extended families (Drooker, 2000; Hanson, 1975).

Similar to SunWatch, the burials at Buffalo reveal little evidence for social stratification. Age and sex were the only significant categories by which grave goods varied. Among adults, more men than women had grave goods that survived interment. Additionally, they were more likely to have objects interpreted as charms, shell ornaments, or pipe bowls, and they averaged higher numbers of such items per burial (Drooker, 2000; Hanson, 1975).

From the original excavations, very little information is reported about subsistence at Buffalo. Indeed, many statements Hanson (1975) presents regarding subsistence begin with “presumably” or “probably”. 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 avian birds were recovered (Hanson, 1975).

Figure 3: Postholes forming the palisade of the "downstream" village, view is facing southeast. From Blatt, 2013, adapted from Hanson, 1975.

Table 1: Site summaries giving phase and occupation time, subsistence economy and diet breadth.

Site	Phase/Period	Occupation Time	Subsistence Economy	Diet Breadth
Duff	Late Archaic	4000-3000 B.P.	Hunting and gathering	Faunal assemblages, nuts, seeds
SunWatch	Fort Ancient	A.D. 1200-1400	Agriculture	Maize, supplementary food stuffs
Buffalo	Fort Ancient	A.D. 1000-1550	Agriculture	Maize? Nuts, fauna

Temporal Changes in Eastern Woodlands Skeletons

Overall, the health indicators at Duff, SunWatch, and Buffalo are consistent with other contemporaneous prehistoric populations. However, there are some patterned differences between the three. This section aims to address the general temporal and regional trends in the health of prehistoric Ohio Valley groups. First is an assessment of various classes of indicators of health from skeletal remains. Additionally, many conditions are age-dependent, that is, the risk changes with age, thereby possibly representing a cumulative risk or an age-specific risk (Goodman and Martin, 2002). As such, accurate aging and sexing of skeletal remains is vital to analysis and comparison of populations.

General and Cumulative Stress Indicators

Growth Rates and Stature

Growth rate is widely recognized as a highly sensitive indicator of health and well-being of a population and is affected by a variety of factors such as genetic influences, growth hormone deficiencies, and psychological stress (Larsen, 1999).

Nevertheless, the predominance of evidence underscores the influence of environment, namely nutrition, on a growing child. While growth is a sensitive indicator of the environment, it is also a nonspecific and cumulative indicator (Goodman and Martin, 2002). Substantial evidence supports the relationship between growth suppression in childhood and attainment of adult body size, including terminal height (Goodman et al., 1984b). Children whose growth was stunted during development will be shorter adults. A problem with assessing stature as a reflection of health is that nutrition and disease have a synergistic relationship whereby poorly nourished juveniles have an increased susceptibility to infection. Disease and infection reduce the ability of the body to absorb essential nutrients needed for survival (Larsen, 1999).

Sciulli and Oberly (2002) found that there was a modest decline in stature over time in Midwestern populations. However, greater stature in Ohio Valley groups occurred in samples inhabiting regions of fertile, high-calcium soils and the differences between populations inhabiting different soils types appeared greater than differences through time. Diaphyseal lengths of the femora of subadults through time were similar. This suggests that stressors most likely to affect femoral length and growth patterns were also affecting populations in a similar manner, regardless of changes in subsistence or population structure. As such, overall discrepancies in stature might be more correlated with local ecological variation rather than changes in diet (Sciulli and Oberly, 2002).

Tooth Size

Like bone, tooth size involves a complex interplay between environment and heredity. Studies show that as much as 80% to 90% of observed covariation in tooth size is due to genetic factors, the remaining is attributed to the environment (Townsend et al., 1994). Through examining the influence of nutrition on tooth size, it was found that poor nutrition will result in smaller tooth size when compared to dietary supplemented individuals (Larsen, 1999). Prehistoric maize agriculturalists have reduced tooth size in both permanent and deciduous dentitions, possibly reflecting an increase in physiological stress due to declines in dietary quality and general health status (Larsen, 1995). Additionally, tooth size reduction is associated with decreased mechanical loading of a softer agricultural diet, resulting from more sophisticated food processing techniques (Sciulli, 1979). At the earliest, starting from the Middle Woodland period onwards, tooth size in Ohio Valley populations was stable, with minor fluctuations in some morphological traits due to genetic drift (Sciulli, 1979).

Markers of Deprivation

Linear Enamel Hypoplasias

Dental enamel provides an invaluable source of information on past morbidity and health because it grows in regular, recognizable patterns and the effects of disruptions to growth are well documented. Although further discussed in length in the Chapter 3, linear enamel hypoplasias (LEH) are grooves or lines marking the point at which enamel development was arrested during the secretion stage of enamel formation (Hillson, 1996). The vast majority of LEH result from systemic metabolic stress, associated with systemic diseases, neonatal disturbances, and nutritional deprivation (Hillson and Bond, 1997). Goodman and Rose (1990) propose that nutritional and disease stress episodes produce hypoplasias only when the magnitude to ameloblast disruption reaches a critical threshold for a given tooth.

The increase in sedentism in rise of agriculture is commonly correlated with an increase in enamel hypoplasia prevalence. The introduction of agriculture is associated with a less varied diet and food sources that lead to a risk of seasonal deficiency, therefore contributing to dietary stress during growth (Larsen, 1999). However, enamel hypoplasias are a nonspecific indicator of physiological stress that may or may not be nutritional stress, therefore its presence or absence cannot be used to make assumptions about diet or malnutrition in a population.

Analyses by Sciulli and Oberly (2002) indicate that hypoplasias in all temporal phases of the Eastern Woodlands are associated with growth depression and stature reduction. Indeed, in a case study at Dickson Mounds, Illinois, dental defects are consistent with other indicators of nutritional stress (Goodman and Rose, 1990). Essentially, indicators of declining health are temporally associated with the induction of agriculture, habitation of larger settlements, and individuals of lower social status in increasingly hierarchical societies.

Porotic Hyperostosis

Porotic hyperostosis is a descriptive term for lesions on the cranial and flat bones, commonly associated with anemia (Goodman et al., 1984b). Similarly, cribra orbitalia involves lesions on the superior border of the orbits. Both manifest as an expansion of the spongy diploe with a corresponding thinning of the outer dense cortical bone resulting in the appearance of surface porosity.

The alteration in skeletal tissue from anemia is caused by the increase in red blood cell production which takes place in the marrow cavities of long bones and the diploe of flat bones. Since the cranial bones are so thin, they are often affected (Goodman et al., 1984b). Typically, lesions form during childhood episodes of anemia because the marrow cavities in children are filled completely with red marrow (Larsen, 1999). The majority of adult cases of porotic hyperostosis are remodeled and healed. The restriction of active porotic hyperostosis to young juveniles indicates that the effects of anemia on adults in the population cannot be determined. There is wide variation in frequency of orbital versus nonorbital lesions across human populations but clinical and paleopathological evidence indicates a common etiology between them (Larsen, 1999). Subsequently, both are subsumed under porotic hyperostosis.

While there are numerous potential causes, nutritional anemia is suggested to be the primary factor in the etiology of porotic hyperostosis for the majority of the documented cases in prehistory (Goodman and Martin, 2002). Specifically, porotic hyperostosis in the New World is most likely related to iron-deficiency anemia since other explanations (malaria, hemoglobin-derived anemias) cannot be applied to these populations (Goodman et al., 1984b). Rates of porotic hyperostosis are interpreted with caution as several other factors, such as infectious diseases and parasites, can affect the occurrence of anemia (Goodman and Martin, 2002). Nevertheless, porotic hyperostosis is commonly linked to the over-reliance on maize agriculture and associated nutritional deficiency (Cook, 1984; Goodman et al., 1984). Variation in frequencies and severity of porotic hyperostosis also existed between sex and social class. This is extremely evident at Cahokia, a Mississippian site where the elite social stratum had differential access to a range of prestige items and higher quality foods, thereby exhibiting fewer health deficiencies (Fowler et al., 1999).

Ohio Valley

Perzigian et al. (1984) and Cassidy (1984) study health and diet transitions respectively from Archaic to Fort Ancient times in the Ohio Valley, and found that a consideration of sociocultural change is highly warranted before making definite conclusions about health or subsistence. The health trends summarized above indicate that late agricultural populations were less healthy than earlier groups. Cassidy (1984) suggested that Archaic and Early Woodlands populations are not readily distinguishable from one another with regard to health although their diet and subsistence practices were different. In contrast, Late Woodlands and Fort Ancient populations are distinguishable by health indicators, despite their similar diets. Therefore, it seems that in this area, there exists a lack of change in health during a transition from foraging to a largely cultivated diet. A striking drop in health follows either during or after the adoption of perhaps a different kind of cultivated diet (Cassidy, 1984).

Perzigian and coworkers (1984) support this conclusion by presenting skeletal evidence that shows a decline in health and nutrition resulting from the abandonment of hunting and gathering and the subsequent development of focal agriculture. Data presented here may be interpreted to show some gains in nutrition and health for the Early and Late Woodlands people over that of the Late Archaic. The same cannot be said of Fort Ancient groups. Dental and skeletal health appear to decline over time as evidenced by an increase in caries, enamel hypoplasia, and skeletal lesions (Perzigian et al., 1984).

The greatest difference in pathological conditions in populations over time in the Ohio Valley is in acquired dental pathologies. Maize agriculturalists exhibit higher frequencies of caries and increased frequencies of abscesses and missing teeth (Sciulli and Oberly, 2002). While in all populations, dental pathologies are a by-product of the normal aging process among agriculturalists, increased dietary carbohydrate resulted in earlier appearance and elevated frequencies of dental pathology. The increased reliance on carbohydrates is also likely associated with the appearance of anemia and infection earlier in childhood in maize agricultural populations (Sciulli and Oberly, 2002). These combined trends in illnesses mutually reinforce each other and presumably reflect a growing synergism among diet, population density, and disease.

Summary

This chapter reviewed the biocultural background, major cultural innovations, and biological patterns of the target populations and samples used in this project (Table 1). Subsistence economy, diet, mobility, and health were considered because dietary deficiencies and disease are among the causes of LEH. Indeed, the most significant pathology in this population consisted of a higher frequency of LEH in the Late Prehistoric period associated with shorter mean adult stature.

Taken as a whole, the skeletal indicators reviewed above among prehistoric populations in Eastern Woodlands suggest an overall decline in the quality of life associated with agriculture. Evidence supports arguments about the relatively good health and nutrition of hunter-gatherers. Additionally, data suggest that the expansion of early agricultural populations was accomplished in spite of general diminution of both child and adult life expectancy rather than being fueled by increased survivorship.

CHAPTER 3: TEETH AND ENAMEL HYPOPLASIAS

Introduction

The following chapter introduces the anatomy of human dentition. It is essential to understand the utilization of dental microstructures when studying the formation and identification of dental indicators of stress. The chapter begins with a review of the macroscopic anatomy of a tooth, followed by a discussion of the types of enamel defects. Since linear enamel hypoplasias (LEH) are the focus of this study, their etiology, timing during growth, and studies on susceptibility are discussed at length. The chapter concludes with an outline of the capabilities and limitations of LEH.

Tooth Anatomy

Teeth are comprised of a crown and a root. White enamel makes up the tooth crown. While it is the hardest biological substance known, since it is not renewable after growth is completed, enamel can be lost throughout life, such as via attrition, or abrasion. By dry weight, mature enamel is 96% inorganic material, 3% water, and 1% organic material (Hillson, 1996). Embedded into the jaws is the root, and the crown projects into the mouth. The pulp chamber of the tooth is surrounded by dentine, forming the bulk of the crown, and projecting into the tooth root, which is coated in a thin layer of cementum (Figure 4). Although still harder than bone, dentine is softer than enamel which is about 30% organic composition (FitzGerald, 2008; Hillson, 1996). The area where the enamel meets the cementum, the cemento-enamel junction (CEJ) only occurs at the point where the tooth crown and root join.

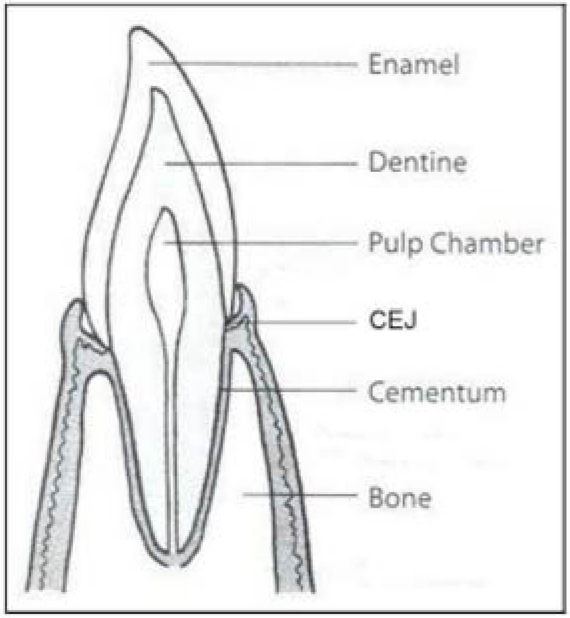

Figure 4: Illustration of tooth anatomy, from White and Folkens, 2005.

Cellular Growth and Structure

The enamel that forms the surface of the tooth is laid down by enamel-forming ameloblast cells in successive layers from the tip of the occlusal end (biting surface) of the tooth, moving apically, and ending at the CEJ (Hassett, 2014). These layers appear in section, similar to the overlapping layers of an onion. Successive growth layers accounting for between 6 and 12 days of enamel formation are separated within the enamel by brown striae of Retzius. These correspond to the circumferential wave-like structures visible on the surface of a tooth, perikymata (Figure 5) (Hillson and Bond, 1997). Perikymata are arranged in a regular pattern of grooves and ridges on the enamel surface in a manner that is not dissimilar from the appearance of accentuated tree rings. As enamel is laid down in successive layers starting at the crown of the tooth, each individual perikymata marks a point of development 6 to 12 days after its nearest occlusal neighbor (Hassett, 2014).

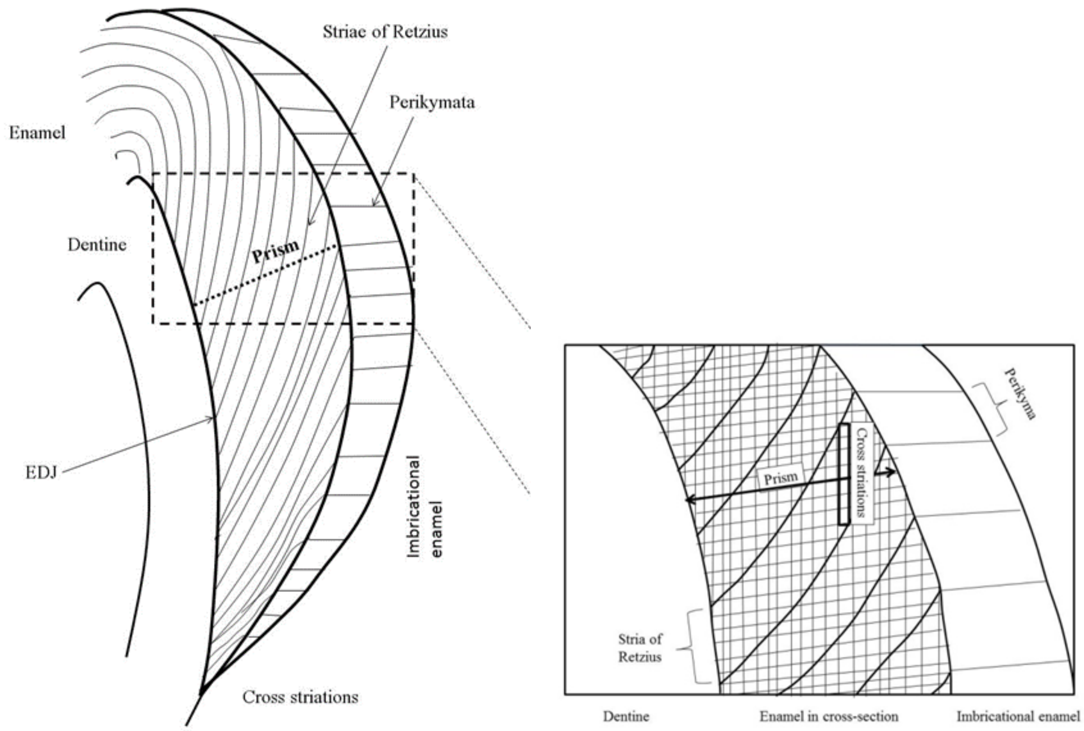

Figure 5: Schematic of the internal and external incremental microstructure of enamel. From Blatt, 2013.

The smallest units of enamel are ribbon-like carbonatoapatite crystallites which are organized into bundles known as enamel prisms (Hillson, 1996). These are the fundamental organizational units of enamel that mark the pathway of ameloblast cells as they make their way from the enamel-dentine junction to the tooth surface (Aiello and Dean, 2002). The course of prisms through the enamel is not straight, rather they appear in alternating zones around the circumference of the crown (Hillson, 1996). This phenomenon is known as decussation, and produces features that can be seen with the naked eye in radially fractured enamel. The decussating pattern of prisms gives strength to the enamel, allowing for an improved function of grinding or cutting surfaces (Hillson, 2005).

By way of imaging from a scanning electron microscope (SEM), the diameters of enamel prisms have regular swelling and constriction (Aiello and Dean, 2002; Dean, 1987; Hillson, 2005). Under light microscope or polarizing light microscope, these constrictions appear as dark and bright lines producing regular sections along the prisms, known as prism cross striations (Figure 6). Measurement of enamel formation and age estimation to the day of dental disruption or death (in subadults) is possible given these cross striations as they represent 24-hour periods of growth (i.e. Blatt, 2013; Li and Risnes, 2004; Osborn, 1981; Smith, 2004).

Evidence for the circadian rhythm of cross striations is well founded (Okada, 1943; massler and Schour, 1946; Bromage, 1989, 1991; FitzGerald, 1998; Smith, 2004; Lacruz et al., 2012) and are near universal in living organisms (Hastings, 1997). For example, when two pig-tailed macaques were administered fluorescent labeling compounds at known intervals, counts of cross striations between the labels precisely mated the intervals between injections (Bromage, 1989; 1991). Smith (2004, 2006) reported on a similar study involving the injection of 17 macaques three to five times with multiple dyes, which further verified the 24-hour regularity of cross striations.

During periods of physiological disturbance in which accentuated striae (which appear as darker or wider lines) are created and enamel defects formed (discussed below), the circadian rhythm continues (Hillson et al., 1999). For instance, the periodicity of enamel cross striations were tested in the permanent dentition of 5 children from a 19th century crypt in London, whose age-at-death was independently known (Antoine et al., 2009). On the teeth of one of the children, developmental defects were present, but the cross striation count for the dentition as a whole match the known age of this individual.

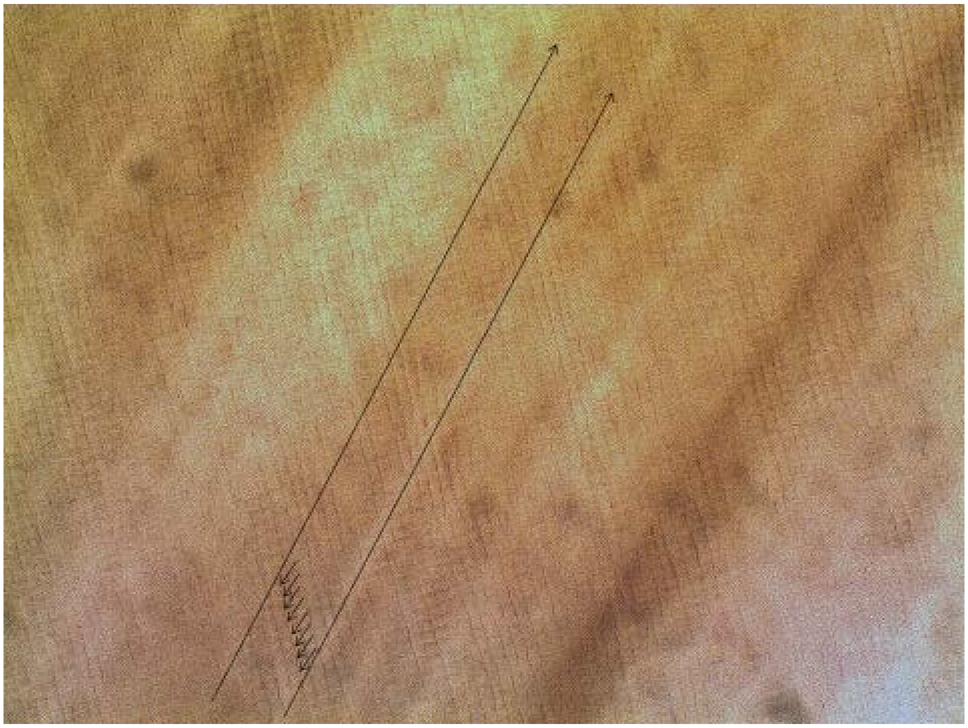

Figure 6: Polarized light image (40x) of cross striations, which are the tiny squares (indicated by short arrows) contained within striae of Retzius (indicated by long arrows). They bisect and run the length of prisms, which are running from the bottom right to upper left corners of the image. Taken from Blatt, 2013.

Enamel Defects

Normal tooth enamel formation can be interrupted by many variables (i.e. stressors) such as birth (resulting in the microscopic "neonatal line"), weaning, nutritional deficiency, subsistence changes, illness, and low socioeconomic status (Bogin, 1999; Hoover, 2001; Larsen, 1999). Disturbances of enamel matrix production can range from a short-term cessation of depositional activity to a permanent interruption in enamel deposition. Internally, these disruptions correspond to defects known as Wilson bands (Simpson, 1997), or rather, irregularities in the striae of Retzius, which may result from short periods of metabolic disturbance (Condon, 1981; Wright, 1990), or from a mild stress event (Massler et al., 1941; Skinner and Goodman, 1992). Despite severity or duration, all physiological stressors leave Wilson bands (Fitzgerald and Saunders, 2005).

Regardless of the recovery of ameloblast cells, any disturbance will result in thinned enamel that circumscribes the tooth crown, although not hindering the timing of its development (Hillson 1996). While enamel defects can vary widely in form, there are three basic classifications:

1. Pit-type defects which can vary in size from an interruption to hundreds of ameloblasts to just a small group. They can be found alone or in association with furrow-type defects (Hillson 2005).
2. Plane form defects that expose large areas of brown striae of Retzius.
3. Furrow-type defects, commonly referred to as linear enamel hypoplasias which represent an exaggeration of the perikymata with a greater than normal spacing down their occlusal wall (Hillson 1996).

Of interest to this investigation, linear enamel hypoplasias (LEH) are the most recognizable and common form of enamel defect (Hillson and Bond, 1997). They are defined as grooves or lines recognized through the premature cessation of ameloblast cells (Goodman and Rose, 1990), which creates a deficit of enamel volume and interrupts the normal distribution pattern of the perikymata, most commonly attributed to disease or malnutrition (Ten Cate, 1994; Hillson, 1996). LEH can vary drastically in thickness, ranging from a microscopic line to a broader macroscopic furrow, as perikymata do not develop in the regular fashion during a systemic stress event (Hillson, 2005). In general, hypoplasias represent acute stress lasting from weeks to several months. The result is an increased distance between successive perikymata, therefore hypoplasia widths represent a quantification of duration of stress events (Hillson, 1992). The occlusal wall of the hypoplasia represents the period of time when 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.

Goodman and Rose (1985) have demonstrated that anterior teeth are more susceptible to hypoplastic defects because they are greatly controlled by genetic factors that dictate their development and thus have limited responses to environmental perturbations. For that reason, many studies centered on enamel hypoplasias focus on permanent incisors and canines in order to yield the maximum number of LEH during childhood (e.g. Guatelli-Steinberg et al., 2004; King et al., 2005; Temple et al., 2012). Specifically, Goodman and Rose (1990) recommend the examination of the permanent maxillary central incisors and the mandibular canines over their deciduous counterparts because perikymata are not as visible. Since deciduous teeth begin forming *in utero*, enamel defects reflect growth disruptions perinatally and are more representative of the mother's instead of a child's health (Griffin and Donlon, 2009).

Nonetheless, limiting the study of defects to only these teeth restricts the interval of development that can be investigated. Permanent maxillary central incisors and mandibular canines near enamel completion around the age of 7 years (± 2 years) (White and Folkens, 2005) and have overlapping periods of crown formation (Reid and Dean, 2000). This excludes information about older subadults, notably when compared to the third molar, which does not complete crown formation until 15 years (± 3 years). Due to the limitations of excluding some teeth from analysis, some researchers have investigated the prevalence of linear enamel hypoplasias on posterior teeth, often finding that some enamel defects observed in anterior teeth are not identifiable in molars due to appositional wear of the crown surface. (Cook, 1984; Cucina et al., 2006; King et al., 2002; Lovell and Whyte, 1999).

Etiology of Linear Enamel Hypoplasia

The etiology of enamel defects is widely debated, but most researchers agree that temporary developmental disruptions during tooth crown formation can result in enamel hypoplasias (Goodman et al., 1984; Goodman and Rose, 1991). Defects can arise due to hereditary abnormalities (involving the entire crown), local trauma (appearing on a single tooth or adjacent teeth), or metabolic stress (Pindborg, 1970; Winter and Brook, 1975). The vast majority of LEH result from systemic metabolic stress (Goodman and Armelagos, 1985; Goodman and Rose, 1990; Hillson, 1996; Larsen, 1999; Skinner and Goodman, 1992).

Preliminary research on hypoplasias involved laboratory animal experiments, in which Mellanby (1929; 1930; 1934) and Klein (1945) related vitamin A or D deficiency to enamel defects. Non-dietary variables were also introduced through experiments involving induced diabetes (Kreshover et al., 1953) and fever (Kreshover and Clough, 1953) in pregnant rats. Results indicate that these factors interrupted enamel formation in their offspring. Amelogenesis was also disrupted in rats inoculated with pathogenic viruses (Kreshover et al., 1954; Kreshover and Hancock, 1956) or bacteria (Kreshover, 1944. Life-history events have also been linked to disrupted enamel formation in a study of a female macaque where defects coincided with her birth, the later removal of all males from her social group, and the subsequent removal of her mother (Bowman, 1991). The latter two associations suggest that psychological stress can produce enamel defects (Guatelli-Steinberg, 2001).

Previous research also strongly links hypoplastic defects to nutritional deficiencies and disease (e.g. Goodman and Armelagos, 1989; Zhou, 1995). Clinical studies on humans have made correlations between defects and a number of systemic and physiological stressors. Higher instances of enamel defects were seen in malnourished individuals and those with micronutrient deficiencies (Infante and Gillespie 1977; Sweeney et al. 1971; Goodman et al., 1991), and it is positively correlated with a variety of diseases including rubella, tetanus, and syphilis (e.g. Pindborg 1982). Temple (2010) documented patterns of systemic stress during the agricultural transition in prehistoric Japan, finding that there are greater frequencies of teeth affected by LEH among agriculturalists when compared to foragers. Evidence from two known age-at-death and sex populations from postmedieval London revealed differences in enamel hypoplasias between sexes (King et al., 2005). Additionally, individuals in this study who died younger in life had an earlier age at first occurrence of enamel hypoplasia than those who survived to an older age.

Analyses of enamel hypoplasias have also been conducted on non-human primates (e.g. Guatelli-Steinberg, 2003; Guatelli-Steinberg and Lukacs, 1999; Miles and Grigson, 1990; Newell et al., 2006; Smith, 2004) and early hominids (e.g. White, 1978). Guatelli-Steinberg et al. (2012) compared the presence of LEH in several species of non-human primates, including *P. troglodytes*, *P. paniscus, G. geringei*, *G. gorilla,* and others. Results indicate that variation in LEH expression across great ape species is not influenced by the taxonomically distinct enamel formation times. With that in mind, etiology of LEH in non-human primates is not dissimilar from causes of physiological growth disruption in humans. Indeed, LEH comparisons can be made between modern and extinct human populations. For example, Guatelli-Steinberg et al. (2005) compared dental defects of Inuit foragers with those of Neandertals. It was concluded that Inuit defects may represent longer growth disruptions than Neandertal defects.

Studies in the Americas have largely focused on the transition from hunting and gathering to agriculture, many of which have suggested that the transition to sedentism and agriculture is associated with the increase in the prevalence of enamel defects (e.g. Cook 1984; Goodman et al., 1980, 1984a; Hutchinson and Larsen, 1988; Larsen, 1995; Rose, 1977; Sciulli, 1977, 1978). The decrease in the quality of diet is unlikely the only cause of the increased physiological growth disruption. Characteristics commonly correlated with agricultural societies may explain the increase in hypoplastic defects. This includes spread of disease from an increased population density, decline of hygienic conditions, and food shortages in the case of drought and crop failure (Diamond, 1997; Stock and Pinhasi. 2010). In addition, the relationship between illness and malnutrition is symbiotic in its effect on physiological stress (Goodman et al., 1984a). Poor nutrition can significantly decrease one's ability to cope with stressful conditions that predispose oneself to illness, further leading to nutritional deficiencies.

Environmental stressors can also be considered, following D'Anastasio et al. (2013), which found a peak in LEH prevalence across the sample population coinciding with the earthquake in 62 AD in Herculaneum, Italy. In another example, at missions in Spanish Florida, limited water resources were contaminated by parasites and microbes, leading to a dramatic increase in the prevalence of porotic hyperostosis and enamel defect (Larsen et al., 1992). Essentially, enamel hypoplasia is a generally nonspecific phenomenon and can be related to a variety of local and systemic disturbances and therefore cannot be connected with specific causal mechanisms (Kreshover, 1960), but specific times of growth can be determined and therefore coincide with seasonal or historical events.

Timing of Linear Enamel Hypoplasia

Due to the predictable rate and method of enamel deposition, LEH represents a unique insight into physiological stress because the timing of dental disruptions, and subsequently the stress event, can be determined. Approximate age at occurrence of the stress event has been estimated by measuring the position of macroscopic enamel defects on the crown surface (e.g. Buikstra and Ubelaker 1994; Goodman and Rose 1990; Rose et al., 1985).

Several different methods exist to evaluate the timing of LEH. The first suggested measuring the distance between the CEJ and the most occlusal portion of the defect which represents the onset of physiological disturbance (Swardstedt, 1966). A dental eruption diagram from Massler et al. (1941) was then used to convert measurements into ages at occurrence. Buikstra and Ubelaker (1994) argued that the distance from the occlusal tip to the defect provides more accurate estimations of age, yet possible attrition to the occlusal surface renders Swardstedt’s (1966) method more practical. This method was later modified by Goodman and coworkers (1980) by producing a table of crown formation ages that were converted from a measure of the distance of each defect from the CEJ by applying mean crown heights to Massler and colleagues’ (1941) chart. This has been recommended as the data collection standard (Buikstra and Ubelaker, 1994).

Although it is commonly used, many researchers have suggested that the chronology can be incorrect due to differences in growth between the sexes and between different demographic groups (Hillson, 1992b; Lovell and Whyte, 1999; Ritzman et al., 2008). An inherent problem in using the methods proposed by Massler et al. (1941) and later modified (Goodman et al., 1980) is that they assume a constant linear growth rate for tooth crowns (King et al., 2002). In reality, varying rates have been noted between individuals, populations, and stages of development (Goodman and Song, 1999; Hillson, 1992a; Hillson and Bond, 1997; Reid and Dean, 2000).

To prove the variability in estimating the timing of LEH, Ritzman and colleagues (2008) tested three methods: the chart method (above, after Buikstra and Ubelaker, 1994), regression equations (after Goodman and Rose, 1990), and histological data (after Reid and Dean, 2006). The regression equations used by Goodman and Rose (1990) were based on the mean crown heights from the standard of Massler et al. (1941) and Swardstedt (1966) where velocity is based on the rate of enamel calcification, which was established by Massler et al. (1941). Reid and Dean's (2000) histological method involved dividing tooth crowns into equal tenths and then calculate the number of days it would take for each tenth to grow. Calculated ages at crown completion was acquired by summing the ten calculations, adding an estimate for the number of days required for cuspal enamel formation, then adding the number of days between birth and crown initiation. This method was applied to large samples from different location in a study conducted by Reid and Dean (2006).

The tests demonstrated that the histological method produced significantly higher ages estimates than the two other methods (Ritzman et al., 2008). However, in using the chart method on modern population samples, it produced age estimates considerably younger than the precise ages and duration of stress episodes obtained through microstructural analysis of archaeological samples (Ritzman et al., 2008). These variations can greatly affect how biological anthropologists interpret early childhood events.

As a response to the appropriateness of applying modern dental eruption patterns to archaeological populations and the problems regarding non-linear rates of enamel deposition, many researchers (such as Fitzgerald et al., 2006; Guatelli-Steinberg et al., 2012; Reid and Dean, 2006; Witzel et al., 2008) have turned to the use of histological analyses to determine the timing of physiological stressors. This involves the examination of a tooth or tooth sections under a microscope in order to observe the enamel microstructures (i.e. perikymata, cross striations, and striae of Retzius). By counting the internal brown striae of Retzius or the external perikymata, when periodicity between these lines is determined, each defect can be assigned to an age at which it occurred.

Histological analysis is capable of providing precise estimates of the timing and duration of stress events (FitzGerald et al., 2006). When teeth are section for histological microscopic examination, internal enamel defects (Wilson bands) appear accentuated, and therefore distinguishable, from normal brown striae of Retzius (SR) (Gustafson and Gustafson, 1967; Rose et al., 1978; Wilson and Schroff, 1970). The brown striae serve as incremental growth structures by which defect occurrence can be analyzed twice a week (FitzGerald et al., 2006). This strongly contrasts the aforementioned chart method (Goodman et al., 1980), which utilized biyearly intervals. The primary disadvantage of histological analysis is the destruction of specimens in order to view the internal structures of the tooth, and also the labor intensity involved to produce a viable sample size.

Conversely, perikymata are the external representations of SR and can be microscopically examined without any destructive means. Utilization of perikymata (discussed above) to study timing and duration of LEH is now widely used by researchers due to its accuracy and non-destructive methods (Guatelli-Steinberg 2008; Guatelli-Steinberg et al., 2004; King et al., 2005; Temple, 2010; Temple et al., 2012). Still, if sectioning is not performed, researchers must estimate or provide a possible range for the periodicity between perikymata, which can skew the estimations of age.

Susceptibility

Hypoplastic defects are found on all tooth crowns, however differences in susceptibility may be caused by age, sex, and tooth type (e.g. Goodman and Armelagos, 1985; Goodman et al., 1987; Hillier and Craig, 1992; Massler et al., 1941; Slaus, 2000, 2008). Enamel hypoplasias are more likely to occur during the first 10 months of life than during any other time of tooth enamel formation because this is a time when metabolic and cellular activities are very susceptible to disturbances (Massler et al., 1941). Additionally, children were found to be more susceptible to physiological stressors around 2.5 years, and 5 years when compared to other developmental time periods. Massler and colleagues (1941) suggest that these susceptible periods in the metabolism of a growing child may be linked to the dramatic increases in growth rates during this time.

Studies on hypoplastic defects linked to sex-related patterns of susceptibility have produced varying results. Goodman and coworkers (1987) conducted a study of permanent dentition in rural Mexican children, finding a higher frequency of females affected. They attributed this to differential access to resources based on sex. Slaus (2000) found a similar result in his study of a Late Medieval Croatian population. This pattern was affirmed by the historic record indicating that females were often subjected to greater levels of stress than males, such as differential access to resources, which may have contributed to sex-related patterns of vulnerability to enamel defects. In contrast, Slaus (2008) found that earlier populations in Croatia saw the reverse pattern. Instead, males and subadults were more susceptible to physiological disturbances such as cribra orbitalia (a skeletal marker of iron deficiency, discussed in Chapter 2) and LEH than females. He attributed these differences to changing local cultural, socio-economic, and political conditions (Slaus, 2008). Other researchers have found no statistically significant differences in susceptibility between the sexes (e.g. Griffin and Donlon, 2009; Guatelli-Steinberg and Lukacs, 1999; Hoover et al., 2005). As such, it appears as though sex-related patterns of susceptibility vary widely and are the result of socio-cultural diversity instead of biological differences.

Certain tooth types are more susceptible to growth disruption during crown development than others. For example, Marks and Rose (1985) found that canines exhibited greater variability of hypoplastic defects when compared to premolars. Goodman and Armelagos (1985) presented supplemental results from their study on prehistoric Native Americans where anterior teeth were more susceptible to environmental insult when compared to posterior teeth. Specifically, they found that maxillary central incisors are more susceptible at early periods of tooth crown development and mandibular canines at later periods of crown formation. Additionally, Goodman and Armelagos (1985) suggested that anterior teeth demonstrate a greater tendency toward hypoplastic defect due to the strong influence of genetics over the size and shape of these teeth. Since teeth under greater genetic control may be less able to alter their size and developmental timing in response to physiological stress, the formation of enamel defects may be the only response available (Goodman and Armelagos, 1985). It has also been suggested that teeth with longer periods of crown development, such as canines, are more likely to form enamel hypoplasias due to their slower rates of enamel deposition (Hillier and Craig, 1992).

Using LEH to Reconstruct Past Health: Capabilities and Limitations

Reconstructing past health is a challenging endeavor, but the analysis of LEH has several advantages compared to other skeletal indicators of stress. First, teeth are ideal specimens to study because tooth enamel undergoes very little diagenetic change postmortem. Second, microscopic and macroscopic LEH analysis rely on nondestructive methods, which are more feasible when studying archaeological specimens. Perhaps one of the most important advantages is the fact that, unlike bone, chronologically developing enamel does not remodel (Goodman and Rose, 1990). As such, teeth are perfect archives of developmental stress during childhood. Once defect locations are measured, growth disruptions can be timed and subsequently converted to estimates of age following and microstructural periodicity standardized developmental schedules (i.e. Ubelaker, 1987).

Despite these inherent benefits of LEH analysis, there are some limitations that hinder the study of overall health in a population. Enamel deposition occurs at varying rates in different areas of both deciduous (FitzGerald and Saunders, 2005) and permanent dentition (FitzGerald, 1995; Hillson and Bond, 1997; Reid and Dean, 2000), making it difficult to identify and count perikymata. Furthermore, appositional enamel on occlusal surfaces covers enamel defects situated in previously deposited layers (King et al., 2002).

Layers of enamel during early stages of deposition are juxtaposed on previous layers, while deposits later in crown development only partially cover earlier enamel layers.

Another limitation is that enamel hypoplasias are nonspecific stress indicators and are only useful to make general observations and comparisons regarding stress (Hillson, 1992a). Therefore, even if an individual's health history is known, it is difficult to match one's medical history to his nonspecific stress response (e.g. Sarnat, 1940; Sarnat and Schour, 1941). The generalized response of hypoplasia formation cannot necessarily be connected with specific causal mechanisms (Hillson, 2005). Implications of the osteological paradox suggest that hypoplastic defects represent an individual's ability to cope with biological and physiological stress (Wood et al., 1992). Instead of serving as an indication of poor health, these defects could therefore demonstrate individual resilience to environmental pressures (Goodman and Martin, 2002). In other words, in order for enamel to exhibit a defect, an individual must be able to survive the incident, suggesting his or her body was receiving sufficient nutrition etc. for it to fight off disease/infection/malnutrition.

One of the biggest concerns is the difficulty presented by tooth wear resulting from physical and chemical abrasion. This is typically a problem when studying permanent, adult dentition. The tooth crown wears as the enamel surface of a tooth comes into contact with the physical environment. As a result, perikymata gradually wear away by means of this erosion and enamel hypoplasias can become indecipherable, although there are methods to compensate (e.g. regression equations after Goodman and Rose, 1990; or developmental deciles of Reid and Dean, 2000).

Despite the inability of enamel hypoplasias to indicate specific causes of stress, the examination of their prevalence, frequency, and duration can reveal valuable information about differential health status between or within a population that is not available in any other skeletal form.

Summary

This chapter introduced the macroscopic and microscopic anatomy of a tooth, specifically those structures involved in dental defects and the evaluation of age from teeth. A review was presented of the various enamel defects, emphasizing linear enamel hypoplasias (LEH) due to their importance in this investigation. The association of LEH as a general indicator of stress has been thoroughly established through the use of contemporary and bioarchaeological research on humans, non-human primates, as well as other animals. In order to assess the timing of LEH, studies have utilized several different methods, but due to the importance of accurate age estimation, the evaluation of histological microstructures of teeth are preferred though not always feasible in field settings. Research has found that susceptibility to enamel growth disruption is dependent on tooth type, age, and crown development, while sex-related patterns of susceptibility are largely the result of socio-cultural diversity. The utilization of LEH has many capabilities and limitations, especially since all studies regarding the reconstruction of past health is underscored by the difficulties presented in the osteological paradox (Wood et al., 1992).

CHAPTER 4: MATERIALS AND METHODS

Introduction

This chapter describes the materials and methods used to test the hypotheses of the current study. First discussed is the sampling procedures and characteristics of the human remains utilized for this study. Second, a detailed presentation is given of the methodological protocol that is necessary to examine linear enamel hypoplasias in this sample. This includes a comprehensive explanation for the creation of the photomontages, followed by a description of how LEH were identified and quantified. Third, the parameters by which variables were measure is described, and the statistical methods employed to test the hypotheses are addressed.

Materials

The human remains used in this study were drawn from three archaeological assemblages in the Ohio Valley, for a total of 40 individuals. The sample consists of epoxy cast replicas of the adult dentition of children from these assemblages. Specifically, these sites consist of the Duff (n=9), and the SunWatch (n = 13) and Buffalo (n = 18) sites as representative of terminal Late Archaic and Late Prehistoric periods respectively, the contexts of which are discussed in Chapter 2. Poor preservation of subadult remains in the archaeological record limited sample size. but the sites chosen for study were dictated by a significant degree of genetic homogeneity (Sciulli, 1990; Sciulli and Oberly, 2002), thereby reducing the potential for variability in data due to genetic factors. With that in mind, it is assumed that any discrepancies in dental growth and development among these populations is attributable to individual variation and/or environmental influences.

In addition to the age distribution of well-preserved subadults, the series was chosen because of regional consistency and the well-studied archaeological and biological contexts. Deciduous teeth are excluded from this study since they do not display perikymata consistently. Teeth that display wear are not included in the sample as they could limit the ability to record full counts of perikymata and therefore skew chronological determinations of death and timing of stress events.

The pre-prepared replicas originate from Blatt (2013), from which the histological ages-at-death of these children were previously determined. The highly accurate dental cellular histology used to determine these ages is capable of estimating age to the day. This provides a sample in which the exact periodicity is known whereby to evaluate the current research questions.

Methods

Goodman and Armelagos (1985) have shown a higher prevalence of enamel defects on anterior teeth (incisors and canines) relative to posterior teeth (premolars and molars), and that perikymata are more prominent and easier to see since anterior teeth are larger and grow quicker than posterior teeth. They suggest that teeth under the greatest amount of genetic control, such as incisors, are the most susceptible to hypoplasias because the frequency of defect by tooth is related to developmental stability. More specifically, anterior teeth are especially controlled by genes governing their development (Dahlberg, 1945), therefore are termed developmentally stable. This characteristic results in different methods of responding to environmental perturbations as compared to less developmentally stable (posterior) teeth. Anterior teeth are less able to alter their size or developmental timing, therefore enamel hypoplasias are the only means of responding to growth disruption (Goodman and Armelagos 1985).

Therefore, because of the differential susceptibility and timing factors associated with hypoplastic defects, data was recorded exclusively using anterior teeth. Goodman and Rose (1990) recommend that LEH studies be limited to the examination of central maxillary incisors and mandibular canines in order to yield the maximum number of LEH as they are the most susceptible. While preference was given to these teeth, preservation conditions limited the number of anterior teeth available for study. For instance, if a central maxillary incisor or mandibular canine was not present, a central mandibular incisor or maxillary canine was used.

The advantage of limiting the analysis to anterior teeth is that these teeth have overlapping crown formation spans. By examining teeth with overlapping periods of crown formation, it is possible to distinguish defects representing systemic growth disruptions from those representing localized disruptions (Hillson, 1996). Once crown growth is complete, development of incremental lines on enamel ceases, therefore to determine age-at-death and duration of linear enamel hypoplasia (LEH), only the perikymata of individuals with incomplete crowns as part of their dentition were counted.

Scanning Electron Microscopy

Attempting to count perikymata via macroscopic methods, such as under a stereomicroscope, or following the standards of Buikstra and Ubelaker (1994) can be difficult and limit the identification of LEH (Hassett, 2014). The surface of the tooth or replica is illuminated by direct light, whose angle is critical and changes down the curved surface of the tooth, obscuring perikymata grooves. Therefore, in order to obtain an accurate count of perikymata and LEH occurrences, it is imperative that counts are taken from photomontages using a scanning electron microscope (SEM). This methodology has become the standard among anthropological studies utilizing perikymata because these microscopes are able to capture images close to the surface of the specimen, therefore minimizing light distortion on the curved surface of a tooth (Guatelli-Steinberg et al., 2005).

The photomontages utilized in this study originate from an investigation by Blatt (2013) in which the same sample was used to evaluate the applicability of the Moorrees method of dental formation to the estimation of age of prehistoric Native American Children. The original digital montages of the labial surface of each tooth replica were created using an FEI NOVA NanoSEM 400 at 50X and set at 5kV (accelerating voltage). It is housed at The Ohio State University Campus Microscopy and Imaging Facility. The electron path in the sample is reduced due to the lower energy of the electrons (low kV), as secondary electrons escape or breakthrough from sharp edges. Therefore fine features can be more accurately represented. The combination of a magnetic immersion lens with low-vacuum technologies delivers fully digital high resolution characterizations of each specimen in an environment that suppresses charge build-up on nonconductive materials. The Nova NanoSEM also suppresses electron-beam induced contamination resulting from previous sample processing steps and is operated entirely by a computer mouse (Blatt, 2013).

In order to maximize shadowing of perikymata grooves, replicas were oriented orthogonally in the microscope's optical axis. A series of 50X images of each specimen were taken to create a digital micrograph photomontage of its surface. A live view of all specimens on the stage was permitted by the four viewing windows on the single monitor within the SEM setup. One window showed the replica under vacuum, two displayed previously captured images and the fourth maintained a live-view of the target sample, simplifying the creation of a photomontage. When necessary, a stub was used rather than tilting the SEM stage, since it was designed to mount a specimen in a 45-90 degree angle. This provided more leverage for the specimens and a more visible shadow cast on perikymata grooves. Overlapping micrographs of each tooth were saved as 8-bit TIFF files, where they can be edited and resaved without any compression loss (Blatt, 2013).

Identifying Linear Enamel Hypoplasias

Using Adobe Photoshop Elements 9, the multiple digital micrographs of each tooth were cropped and overlapped by matching landmarks such as accentuated perikymata, LEH, or pits (Blatt, 2013). Once completed, this created a single image of the entire tooth surface. Image resolution was enhanced by sharpening and adjusting brightness and contrast. Before perikymata were counted, each photomontage was thoroughly examined for the presence of LEH. As discussed in Chapter 3, it is important to note that LEH defects are most objectively identified by increased perikymata spacing. As such, wherever spacing between perikymata grooves increased, it was indicated with a mark on the side, labeling alphabetically, to denote where perikymata counts should begin and end (for example, see Figure7).

Perikymata were counted from the digital images so they could be magnified or adjusted when needed (raw data collection is available from the author upon request). A Wacom Bamboo Create Pen and Touch Tablet was first used to identify LEH, then to mark perikymata in the image as they were counted. Counting started near the occlusal surface and ended toward the cervical end of the tooth or cemento-enamel junction. Perikymata were counted in this fashion until the onset of each defect in order to determine the age at which stress events occurred. Subsequently, perikymata were counted within each accentuation to establish the duration length of each LEH. This procedure eliminated any visual confusion and minimized intra-observer error. Perikymata of each tooth were counted twice at least 1 week apart to evaluate observer reliability. The perikymata number for each count was recorded and the mean perikymata count was used as the final count result.

Following this data collection, LEH were matched between teeth of each individual in order to eliminate any defects associated localized trauma. LEH were matched by comparing the duration, interval between successive defects, and the estimate of the age-at-onset for each hypoplasia on each tooth. The calculation for the latter is described below.

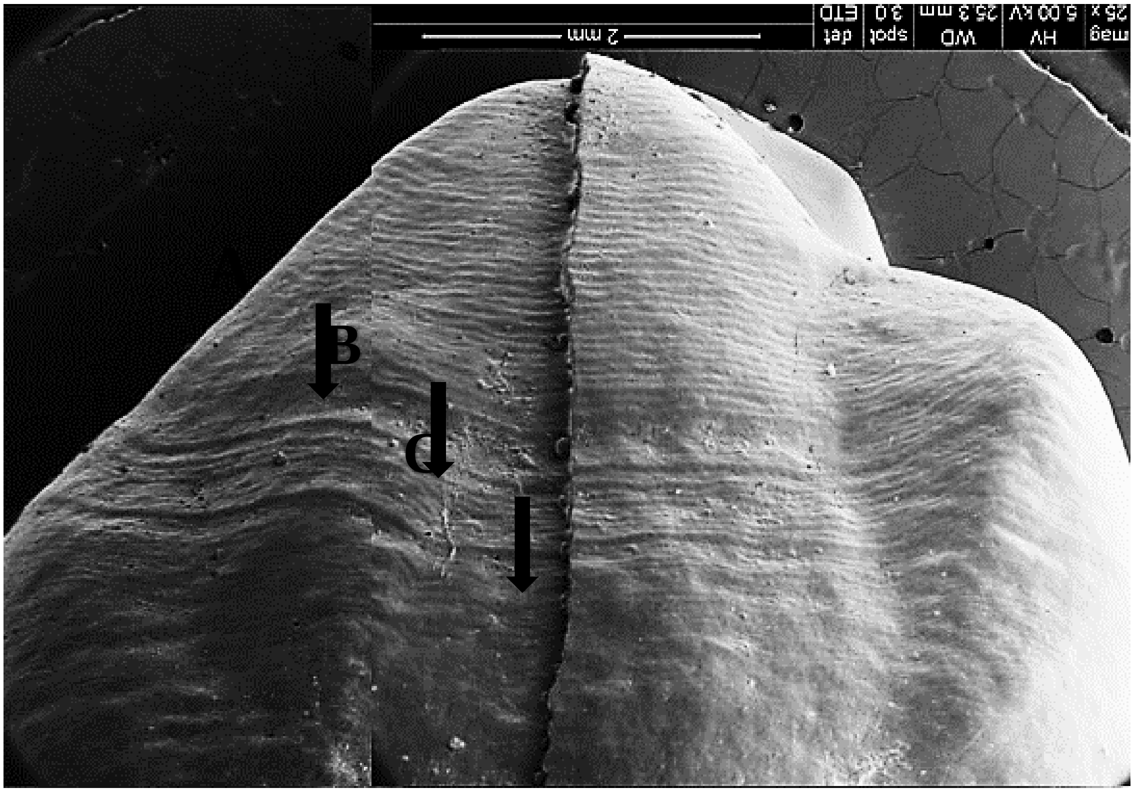

Figure 7: Lower left canine of individual B6-71 from SunWatch with arrows indicating LEH (and accentuation in the horizontal perikymata). LEH A includes 6 perikymata, LEH B and C both have 2 perikymata each. Occlusal surface is towards the top. Modified from Blatt, 2013.

<u>Data Analysis</u>

Following King et al. (2005), several parameters were examined, including frequency, prevalence and duration of growth disruptions. Frequency was calculated for each individual by counting the average number of matched LEH occurrences among the anterior teeth. Individuals with two or more 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 two or more 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. The duration of each LEH episode was recorded by expressing the number of all perikymata affected by a growth disruption.

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 occlusal wall is the sloping wall of the occlusal portion of a groove where perikymata are more widely spaced than they are in the cervical wall. In some cases, the transition between occlusal and cervical walls of defects was not always clear, or the defect was only a few perikymata wide. In which case, it was impossible to differentiate between occlusal and cervical walls. Still, the method employed by Hillson and Bond (1997) was considered and in an attempt to better reflect the duration of growth disruption, the total number of affected perikymata was divided by two. However, when considering many individuals, it was advantageous to count the total number of affected perikymata and use it as an indirect indicator of growth disruption duration (Guatelli-Steinberg et al., 2005). Following this number, stress duration was calculated as the total number of perikymata as observed above multiplied by a factor of eight acting as the true periodicity for the number of days between successive perikymata growth for these populations as calculated by Blatt (2013).

In order 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. While periodicity in humans has been observed to range from 5 to 12 days, a modal periodicity of 8 days was determined the target populations through histological analysis by Blatt (2013). The total was then added the initiation times (Reid and Dean, 2006) and the mean cuspal enamel formation time for each specific tooth (also determined by Blatt, 2013 for these specific populations), then subsequently divided by 365.25 to yield age-at-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 at death (with correction factors) for all individuals in this study, correlated with LEH occurrence and duration, were previously provided through microstructural and histological analyses of Blatt (2013). In this way, many of the estimations normally required for estimation of age of and duration of LEH, were eliminated.

LEH prevalence was compared between populations using a Fisher's exact test. A box plot was produced to depict ranges of stress episodes. Stress episode duration and age at first occurrence was compared between the populations using a one-way ANOVA with Tukey's HSD test for each pairwise comparison. The project tested the similarities and differences in the stress experiences of hunter-gatherers as compared to agriculturalists in the prehistoric Ohio Valley.

Summary

In summary, replicas of anterior immature permanent teeth from three archaeological sites in the Ohio Valley were used to determine the frequency of linear enamel hypoplasia (LEH), the duration of each disruption, and the ages at which each stress episode occurred. Perikymata were imaged using SEM and were counted from micrograph montages of the dental replicas. LEH were identified from these montages, followed by estimates of period of growth disruption and the ages at which each LEH occurred. These variables were compared between samples as an attempt to examine the stress experiences of the three populations from which the samples are derived.

CHAPTER 5: RESULTS

This chapter outlines and displays the results of this study, which are organized by prevalence and frequency of LEH occurrence, duration, and age-at-occurrence. For the purposes of statistical analysis, the 40 individuals originally included in the sample were limited to 32 individuals. Eight were eliminated either due to the presence of only a single anterior tooth, or because the individuals were too young for consideration in that the perikymata were unobservable. Here, a lack of enamel resulted in too few perikymata to compare between teeth.

Prevalence and Frequency of LEH

Of the 15 individuals from Buffalo, 13 (86.7%) presented hypoplastic defects. Prevalence among SunWatch individuals was similar; 8 of the 9 (88.9%) subadults displayed enamel hypoplasias while those from the Duff site exhibited a prevalence of 4 out of 8 (50%). Frequency of LEH between 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. No more than three defects were matched between anterior teeth on any individual from all groups. A summary of these results are given in Table 2.

Despite the differences in prevalence, Fisher's exact tests showed that they did not differ significantly between the three sites, nor between any two ($p > 0.05$). The results of these tests, including the Chi-squared term and p-value are given in Table 3.

Table 2: LEH prevalence and frequency for each site.

Site	Total Number of Individuals	Number of Defects	Average LEH Frequency	Prevalence
Buffalo	15	13	1.8	86.70%
Duff	8	4	1.125	50%
SunWatch	9	8	1.89	88.90%

Table 3: Results of the Fisher's Exact Tests.

Comparison	Chi-Squared Value	P-Vlaue
B, D, S	4.953	0.1189
B, S	0.025	1
D, B	1.98	0.131
D, S	1.496	0.131

Duration of LEH

The duration of stress episodes, given in days, varied largely within each group, the ranges of which can be seen in Figure 8. The boxes represent interquartile ranges of stress episode duration for the samples. The whiskers indicate the 95% confidence intervals, and those stress episodes found above the whiskers are outside this interval. The individuals from the Duff site had a longer average duration than those from Buffalo and SunWatch. Duff individuals endured stress episodes for an average of 45.75 days, whereas those from Buffalo and SunWatch endured an average of 33.04 and 25.22 days respectively. Notably, specimen B45 from the Duff site was omitted for duration analysis, but was included for all other considerations. The duration of the linear enamel defect exhibited on the teeth of B45 (Figures 9 and 10) could not be assessed from methods used in this investigation since perikymata were not visible in the deep furrow. For the remaining 24 individuals, Tukey’s HSD test suggests subadults from the

SunWatch site experienced significantly shorter stress episodes than those from the Duff site, as a population (p = 0.0244). There was no significant difference between Duff and Buffalo (p = 0.1888) nor between SunWatch and Buffalo (p = 0.3269). Conversely, when considering the average duration of LEH for each child, instead of average duration in each population, ANOVA shows no significant difference between samples (p=0.162).

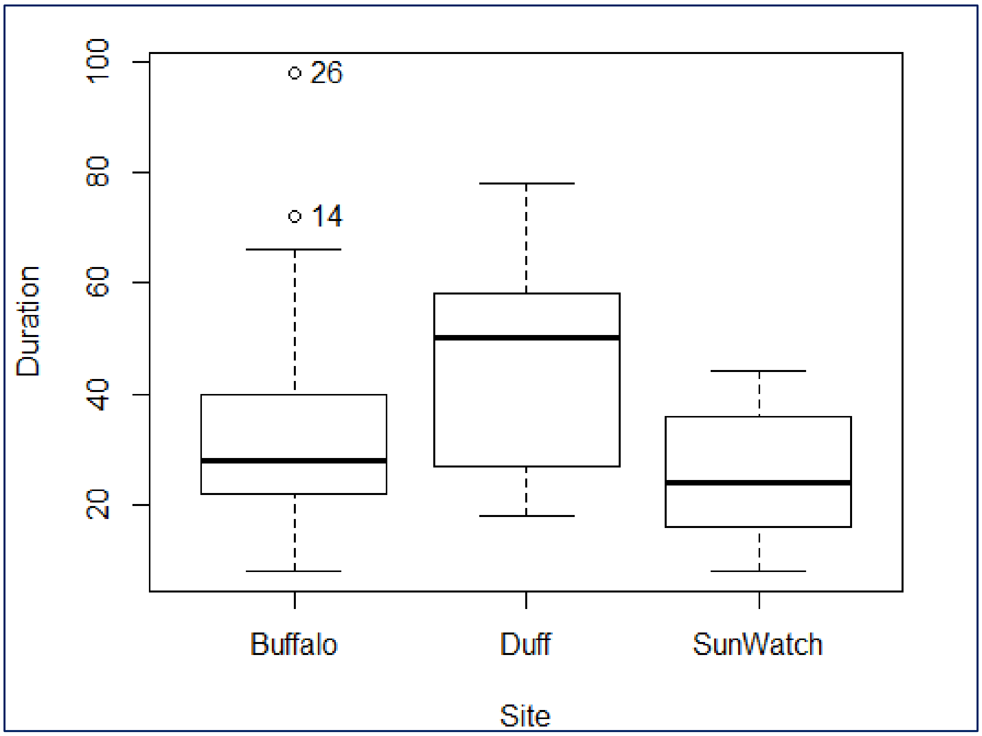

Figure 8: Stress episode duration, given in days.

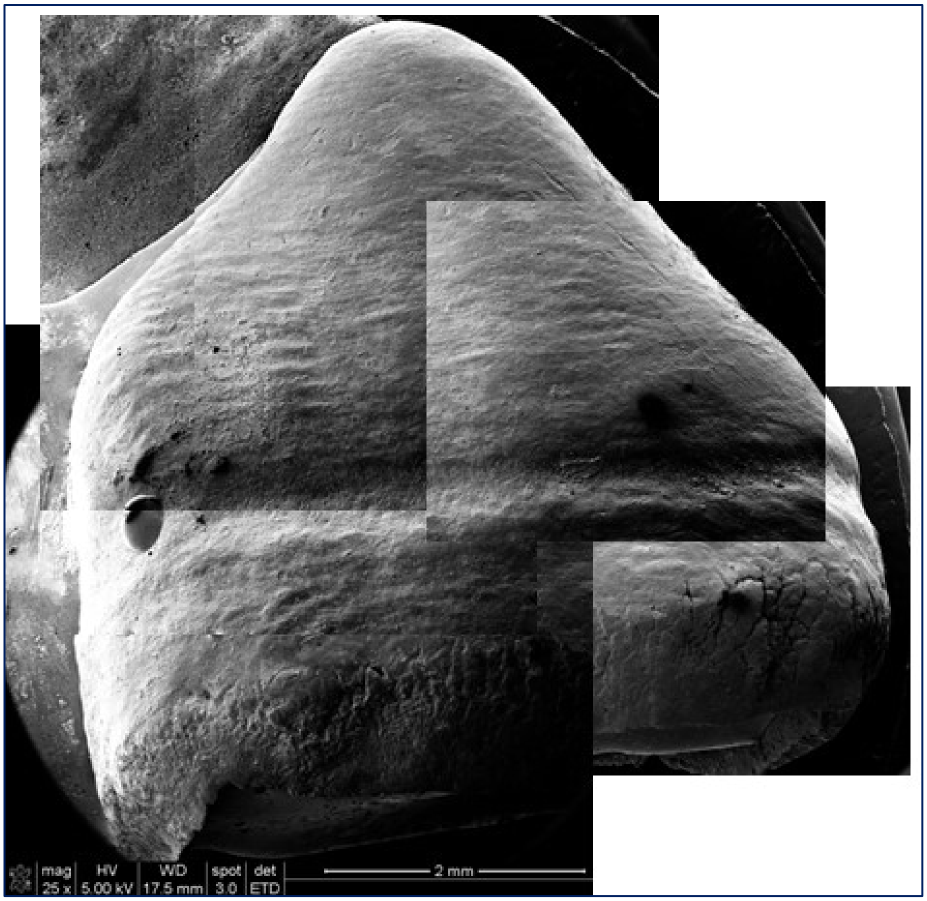

Figure 9: **Lower right canine of B45 from Duff. Note the deep furrow and lack of visible perikymata within defect. From Blatt, 2013.**

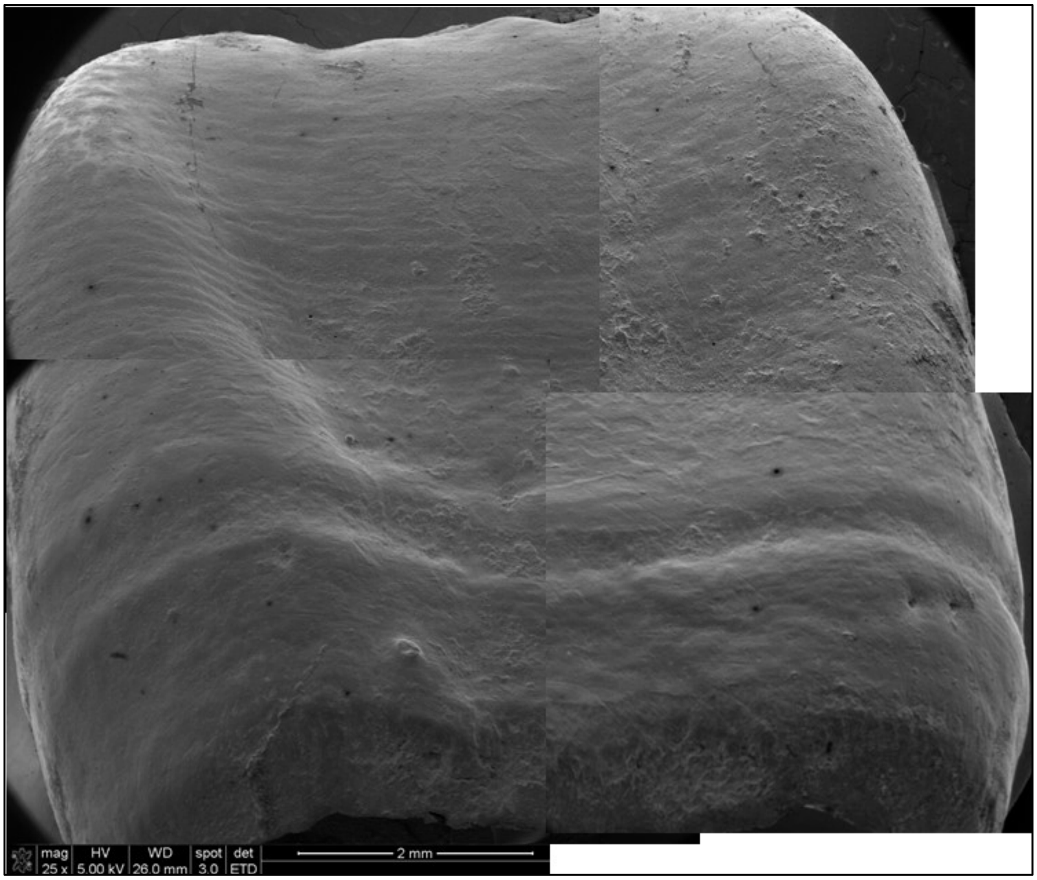

Figure 10: Upper right first incisor of B45 from Duff. Note the deep furrow and lack of visible perikymata within defect. From Blatt, 2013.

Age at Occurrence of LEH

ANOVA indicates that there is no significant difference in age at first occurrence between the three groups ($p = 0.374$). The ages at which subadults from all populations experienced their first stress episode observable in the permanent dentition begin between 0.89 and 2.67 years. Figure 11 shows the trend between the samples in the ages at which the stress events occurred. The values for each site represent the number of individuals who experienced their first, second, or third (LEH A, LEH B, LEH C respectively) stress event in the given age range over the total number of individuals exhibiting that LEH. The consistency of multiple LEH occurrences within the second and third year of life among the SunWatch sample prompted an investigation of the interval between LEH.

The average time between successive LEH are as follows; Buffalo, 155.43 days; Duff, 115.6 days; SunWatch, 86 days.

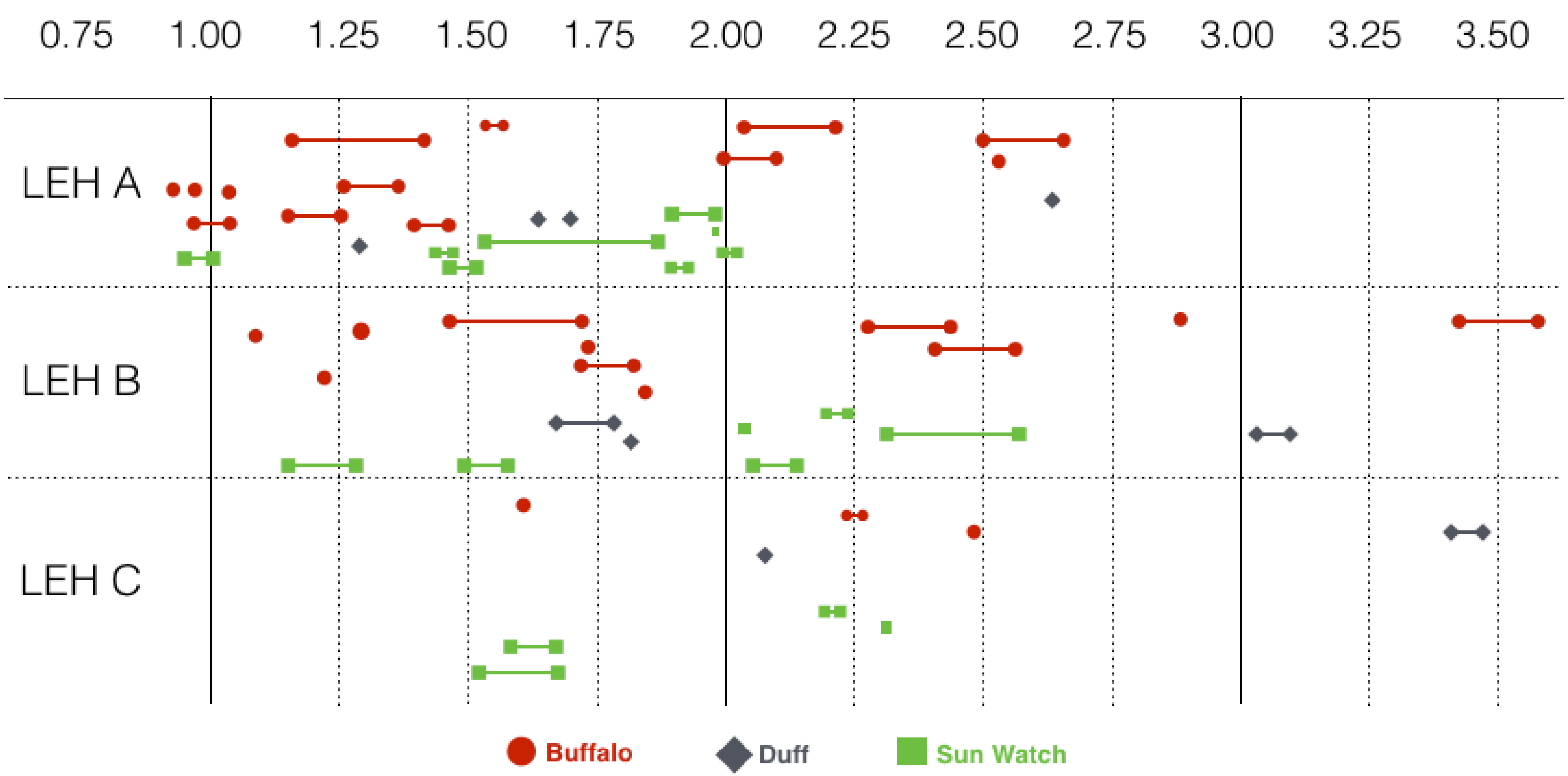

Figure 11: Ages at LEH occurrences by site, where lines represent the range at which stress episode first began, individual points represent the only viable age at which the episode could have occurred.

Summary

Overall, analysis of LEH from the three samples reveals a number of noteworthy findings. First, individuals from the Duff site endured longer stress episodes on average than the other sites, yet had the lowest prevalence and frequency. Second, the longest average interval between stress episodes occurred in the Buffalo site, although subadults from this site shared similar frequency and prevalence with those from SunWatch. Third, SunWatch also had the shortest average duration for each stress episode, as well as the shortest interval between successive episodes.

CHAPTER 6: DISCUSSION

Overview

The following section discusses the data from a comparative perspective of similar studies using other populations. Similarities and differences are compared between the three sites and others in regard to subsistence strategy and diet breadth. This chapter also outlines the limitations that should be recognized when considering the hypotheses of this study.

Although prevalence among the Buffalo and SunWatch children were higher than in the Duff sample, this difference was not significant, suggesting that a similar number of individuals were subjected to experience physiological stress, despite subsistence strategy differences. The average number of defects per tooth showed the lowest frequency among the Duff sample, supporting part of the hypothesis that the agricultural groups suffered a higher frequency of stress episodes. Average duration of LEH defects was significantly different between Duff and Sunwatch, where Duff children suffered longer stress episodes than SunWatch children. Here, the duration analysis refutes the other portion of the hypothesis where foragers in this study endured longer stress episodes than that of maize agriculturalists. Interestingly, this suggests Duff children experienced physiological stress less often than Buffalo or SunWatch children, yet during those occurrences, the stress episodes lasted longer than the other two samples.

Duration, Prevalence, and Frequency

Trend differences in LEH prevalence, frequency, and duration are found because they are measuring different aspects of the stress experience. For example, Larsen and Hutchinson (1992) showed that among post-contact Native Americans of the Georgia Bight, LEH prevalence decreased when compared to earlier foragers, yet stress episode duration increased. In another study, greater stress episode duration was evident in a sample from Point Hope Tigara compared to Neandertals from Krapina, despite similar LEH prevalences (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. Therefore, concordance between LEH prevalence, frequency, and stress episode duration should not be expected, although it is possible.

Instead, variation in the results above may be attributed to differences in subsistence strategy and dietary breadth, and other socio-cultural factors. The dietary breadth of the Duff people focused on diverse faunal assemblages, nut harvesting, wild plants, and riverine resources (Emerson et al., 2009; Parmalee, 1969). 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. This suggests that Duff 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 storage techniques, if climate variations occurred causing food depletion or relocation, Duff individuals may have experienced longer episodes of physiological stress.

Fort Ancient sites, including SunWatch and Buffalo, are characterized by their reliance on maize agriculture (Rossen, 1992). The increased frequency of stress episodes observed in both SunWatch and Buffalo children supports previous studies suggesting an increase in health problems in agricultural populations (for example, Cook, 1984; Danforth, 1999; Larsen, 2006; Temple, 2010). Although maize meets daily caloric requirements, it is deficient in essential amino acids, providing a poor source of protein (Whitney and Rolfes, 2011). Therefore iron-absorption is very low (Ashworth et al., 1973). The nutritive value of maize is altered during the process to transform it into food. Grinding preparation decreases the nutrients by removing important minerals and some fiber (Rylander, 1994). It is often assumed that maize gruel is introduced to infants in Late Prehistoric populations around the age of 6 months, when their growth needs exceed the nutrients supplied in breast milk (Wright, 1997). Even though breast milk is still a significant component of an infant's diet during this time, maize gruel may not be sufficient in providing supplementary nutrients. Therefore, throughout the weaning process, hypoplasias may represent stresses induced from infectious pathogens instead of nutritional deficiency (Blakey et al., 1994).

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). Decreased host resistance is likely to have been a cause of the increase in LEH frequency in the Buffalo and SunWatch subadults. Indeed, Sciulli and Oberly (2002) show that there is a decrease in overall health in these two populations when compared with the Duff sample (and other foraging groups) supported by an increase in skeletal infection and cribra orbitalia.

However, a diminished quality of diet is unlikely to be the only cause of an increase in frequency of stress episodes and infection. Agriculture allows for population growth and sedentism, affording ample opportunity for the introduction and perpetuation of novel pathogens (Goodman et al., 1984a; Larsen, 1995). Additionally, such pathogens and other stressors may be introduced to SunWatch by migrations from other agricultural societies, such as those in the east from Mississippian populations, whose inhabitants suffered high rates of physiological stress and disease (Cook, 2008; Essenpreis, 1978; Goodman et al., 1984a).

Increased maintenance of diseases and other pathogens in agricultural societies compared 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 significant reduction of stress duration suggests SunWatch experienced shorter or less severe episodes of physiological stress. However, the high frequency of LEH in this population in connection with the short interval of time between occurrences implies an overall decline in comparative health.

Ages-at-Occurrence and Age-at-Death

Many previous studies have linked hypoplasias after the first year or so of life to the negative effects of weaning (Coppa et al., 1995; Corruccini et al., 1985; Ogilvie et al., 1989; Ubelaker, 1992; Webb, 1995; and many others). However historical documentation shows a discrepancy between age pattern of hypoplasias and age of weaning (Blakey et al., 1994). As discussed above, other elements leading to enamel defects include nutritional problems, illness, or poor hygiene, therefore weaning is not the sole factor leading to growth arrest in enamel (also see Hassett, 2014; Katzenberg et al., 1996). With that in mind, no assumptions about the onset of weaning can be made regarding the ages at first LEH in the three samples used in this study, especially since there was not a significant difference between them.

In other archaeological samples, it is frequently found that the occurrence of hypoplasias peaked between the ages of two and four years old (Corruccini et al., 1985; Goodman et al., 1980; Hillson, 1979; Hutchinson and Larsen, 1995; Powell, 1988; Storey, 1992). Such an analysis in this investigation cannot be performed due to the young ages at death of the individuals in the samples. Although the timing of crown growth of anterior permanent teeth begins around the first year of life, ending near the 5th, over half the individuals in the original sample (25 out of 40) were under the age of 4 when they died, before crown completion. In that regard, peak frequency cannot be determined since individuals did not live long enough to reflect a time of maximum LEH frequency during growth.

The low mean age at death at the Duff, Buffalo, and SunWatch sites is notable in this study. The average ages for the subadults recovered from each site are 4.89, 3.82, and 2.07 years respectively. Notably among SunWatch children, this reflects a high rate of infant and toddler mortality, which is commonly seen in other Fort Ancient as compared to Archaic populations (Cassidy, 1984).

In regard to the average age at death with frequency, prevalence, and duration of stress episodes of children in this study, the Duff sample exhibits the smallest LEH frequency and prevalence, longest mean duration, but the oldest age at death. In contrast, the SunWatch sample has the largest LEH frequency and prevalence, the shortest duration, and the youngest age at death. For all three categories, Buffalo children display intermediate values. These general trends in health between populations suggests a decline in overall health during the Late Prehistoric. Since diet is a primary difference between Late Archaic and Late Prehistoric populations, the increase in episodes of physiological stress in young Late Prehistoric children may have been the result of the shift to maize agriculture during this time. This is also indirectly reflective of family size and birth spacing; with higher toddler mortality rate among agricultural groups and the use of children in field labor, the incentives are high to increase birth frequency and decrease inter-birth intervals.

It is noteworthy that enamel hypoplasias can be observed only on those subadults who survived the stress events, and cannot be known if the children died during another episode, or due to some other cause. Indeed, markers of disease or deprivation in human remains represents only a fraction of the health in a population since most diseases will leave no lesions on bone, or will abate or kill their hosts before being expressed on skeletal tissue (Wood et al., 1992).

There is evidence to suggest that subadults who are chronically stressed, such as through disease, malnutrition, or illness, are more likely to experience greater levels of morbidity and mortality (e.g., Goodman and Armelagos, 1989). In order to further evaluate morbidity and health among the three populations it is necessary to explore other indicators of health. In fact, Sciulli and Oberly (2002) investigate such variables within these groups by examining other pathological conditions on adult and subadult remains. While many pathological markers were not present on many individuals from these sites, they found stature to be reduced in the populations with higher LEH frequency and prevalence (Buffalo, and SunWatch). This appears to suggest that frequent occurrences of physiological stress during childhood leads to growth rate depression, and therefore shorter attained stature in adulthood. As such, the results of this analysis parallels the conclusions of other studies surrounding health in the Ohio River Valley.

Limitations

Several limitations should be considered within the analysis of this project. First, the most evident issue is the small sample size. Subadult 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 from analysis due to the presence of only one anterior tooth where two were needed to be considered for analysis.

Additionally, enamel deposition is not linear in the permanent dentition (Fitzgerald, 1995; Hillson and Bond, 1997; Reid and Dean 2000). Furthermore, appositional enamel on occlusal surfaces of teeth is argued to hide defects situated in previously deposited layer (King et al., 2002). Layers are laid on top of one another during early stages of enamel deposition, and only partially cover previous layers in later crown development. For example, the occlusal surface of a molar can represent as much as 40-50% of development time, therefore the prevalence and frequency of enamel defects would be underestimated. When only surface enamel is examined, defects that occurred early on in the molar's development would be hidden because they are overlaid by successive enamel layers, thereby reducing the estimates of prevalence and frequency of hypoplasias (King et al., 2002).

Finally, although LEH analysis has been utilized in a number of settings, the paucity of reliable hypoplasia identification in the current literature presents a challenge when using microscopic methods. While LEH identification under a microscope will reveal more defects than via macroscopic methods, especially where perikymata are more densely packed (Hassett, 2014), means of identifying accentuated perikymata are somewhat vague without proper tools which were unavailable for this project, such as a measuring microscope with digital micrometers.

The beginning of a hypoplasia is typically identified as the point where perikymata are more widely spaced than their occlusal neighbors. Yet the spacing between each successive perikymata can be variable through different parts of the same tooth since perikymata are packed more tightly in some areas compared to others (Schwartz et al., 2001). As such, the perikymata spacing are not directly comparable in different regions of the same tooth, and cannot be used to identify the larger gaps where abnormal enamel develops due to growth disruption (Hillson, 1992). In order to *accurately* calibrate the spacing between perikymata across differently packed areas, the mean distance must be calculated based on the nearest neighbors. Then if the moving standard deviation of these is sufficiently different from the normal pattern of distribution, these perikymata should be considered evidence of a growth disruption (Hassett, 2014). A reliable and definitive study of dental enamel via microscopic examination is inordinately time consuming and microscopic analysis requires expensive analytical tools that are impractical in many field and laboratory situations.

Summary

Duration, prevalence, and frequency of linear enamel hypoplasias must be considered within the context of each population from which data were gathered. Conclusions about health cannot be drawn when making comparisons between sites without recognizing the impact of each environment. Seasonal migrations among Duff children reduced the risk of resource depletion and nutritional inadequacies, possibly resulting in a low frequency of observed LEH, and the oldest average age at death. The high frequency and prevalence of LEH among Buffalo and SunWatch populations can be partly attributed to their reliance on maize agriculture and its lack of essential nutrients. The cooperative nature between nutritional deficiency and infectious pathogens allowed for the increased and perpetual maintenance of diseases in these Fort Ancient sites, which may have led to the high rate of death among the toddlers in this study.

CHAPTER 7: CONCLUSION

This thesis has examined childhood health in three skeletal samples from the Ohio River Valley through the analysis of linear enamel hypoplasias on the permanent dentition of subadults. The results of this analysis are interpreted within the archaeological contexts of each sample and were compared between populations to test the similarities and differences in the stress experiences between foragers and agriculturalists. The analysis followed the conclusions of similar studies regarding trends in the overall health of populations during the transition to maize agriculture (e.g. Cook, 1984; Danforth, 1999; Larsen, 2006; Sciulli and Oberly, 2002).

The results of this study indicate that despite small sample sizes there were observable differences in LEH frequency and duration between the Duff, Buffalo, and SunWatch subadults. Indeed, the hypothesis was partially confirmed where the agricultural samples reflected an increase in physiological stress by exhibiting a higher LEH frequency than the foraging population (Table 2). However, 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 addition to differences in subsistence strategy, a possible explanation for the differences in the health experience 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 “infrastructure” was probably in its early stages of development (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 a skeleton, or their teeth, unmarked by acute effects of illness. In these cases, the fatal stress episode would not be recorded in the dentition if ameloblast activity was disrupted for a period longer than 8 days since that is the periodicity between each perikymata.

The concept of a lack of developed infrastructure affecting variation in health between populations appears to be reflected in the health index of all Eastern North American samples (Sciulli and Oberly, 2002). There is a trend for populations with a less-developed infrastructure to exhibit higher scores on the health index, meaning they exhibited fewer pathological conditions on their skeletons and teeth (Steckel et al., 2002). In the sense of physical quality of life, these populations were probably healthier. On average, in populations with a less-developed infrastructure, individuals with obvious effects of disease did not live as long, although they were generally healthier than their counterparts with a well-developed infrastructure. Conversely, the latter populations would have had a better chance of healing their sick people, or at least surviving acute phases of disease. As such, these populations would have had longer lives, and therefore if chronic phases of diseases were to develop, they would manifest in their skeletal remains (Sciulli and Oberly, 2002). It can therefore be argued that foraging populations (including the Duff population) enjoyed a higher child survivorship rates and a higher overall quality of life, whereas agricultural populations (SunWatch and Buffalo) lived longer if they survived toddlerhood.

The purpose of this project was to reconstruct growth disruption from subadult teeth using linear enamel hypoplasia prevalence, age of occurrence, and duration among populations with different subsistence strategies and social structures from the prehistoric Ohio Valley. Analysis of childhood growth disruption serves a proxy for understanding health and stress in archaeological populations since children are the most susceptible to environmental insults. While previous studies show a trend in greater prevalence and duration of stress events in agricultural populations, Temple and colleagues (2013) demonstrated that stress experiences cannot be generalized by subsistence strategy and the comparison of stress prevalences alone. Rather, age-at and duration-of stress events are necessary to construct patterns of health due to multiple bio-cultural factors.

From a methodological point of view, this project was the first to combine analysis of LEH with chronological, histologically-derived ages-at-death from archaeological specimens. This methodology is intended to promote critical discussion within the field of the current limits of assessing LEH patterns (or other skeletal stress indicators) in children without accurate age estimates from non-European populations. It is an attempt to improve not only the bioarchaeological understanding of stress and health in the past, but the variation which exists in human adaptive strategies to environmental and cultural insults on survival.

Future Work

With that in mind, future work on reconstructing past health from dental remains should utilize ages estimated from microscopic methods appropriate for the sample. The addition of enamel defect studies on deciduous teeth from Ohio Valley Native Americans would expand the window of growth that could be analyzed from this population. It would answer questions regarding prenatal care and the health of pregnant mothers, which may differ from the health and physiological stress of women when they are not pregnant. It would also be useful to compare the dental stress markers recorded in this study with skeletal stress markers, as the latter is a product of physiological growth rather than chronological growth that appears in teeth. Future research may utilize the results and analysis of this study when exploring temporal trends in health of Ohio Valley Native Americans as they relate to the social and political influences of the Mississippian culture on the Fort Ancient Late Prehistoric culture.

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