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# Childhood Stress at Rinconada Alta (AD 1470-1532): An Examination of Linear Hypoplastic Enamel Defects on the Central Coast of Peru

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Graduate Program in Anthropology A thesis submitted in partial fulfillment of the requirements for the degree in Master of Arts © Jessica Lacerte 2019

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### ABSTRACT

This MA thesis investigates non-specific childhood stress at Rinconada Alta (Rímac River Valley, Peru) through the analysis of linear enamel hypoplastic defects (LEH). Dental impressions were taken from a sample of teeth from predominantly Incaperiod (AD 1470-1532), Yschma remains with some admixture of Late Intermediate period (AD 900-1470) burials. The sample consists of 10 adult females, 11 adult males, and 5 adolescents of indeterminate sex with fully occluded adult teeth (with the exception of the third molars). This thesis employs Scanning Electron Microscopy (SEM), which increases the visibility of linear defects, to determine the frequency, age, and duration at which metabolic disruption affected enamel growth of the permanent dentition during early childhood. The use of SEM to examine dental enamel in Andean populations is relatively novel, despite this technique being used widely elsewhere in dental anthropology. The secondary aim of this thesis is to compare the results of LEH analysis from Rinconada Alta to several other penecontemporaneous coastal, pre-Columbian sites from the Andean region. Finally, two different equation-based methods of enamel defect age estimation were compared to test the use of these formulae on a sample of South American origin. This research aims to shed new light on childhood stress in pre-Columbian Peru and expand bioarchaeological dental literature by using microscopic (SEM) methods to examine LEH alongside macroscopic methods of defect observation.

KEYWORDS: Andean bioarchaeology, dental anthropology, linear enamel hypoplastic defects, physiological stress, prehistoric health

### SUMMARY FOR LAY AUDIENCE

This MA thesis research examines changes in the frequency and duration of childhood stress (i.e. illness, malnutrition) at the site of Rinconada Alta on the Central coast of Peru. This region was incorporated into the Inca empire around AD 1470 and was occupied by the Yschma culture prior to conquest. The permanent (adult) teeth form during the first few years of life and provide a "snapshot" of childhood health as the enamel forms. Dental casts created from adult and adolescent skeletons from the site of Rinconada Alta will be examined for dental indicators of stress using Scanning Electron Microscopy (SEM). The goal of this research is to use SEM dental analysis to better understand how Inca conquest on the Central coast may have impacted childhood stress at Rinconada Alta. This research broadly contributes to bioarchaeologists' knowledge of prehistoric, Andean lifeways, and is part of a long-term, collaborative bioarchaeological study of the human remains at Rinconada Alta overseen by my supervisor at Western University, Dr. Andrew Nelson.

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#### CHAPTER ONE: INTRODUCTION

Bioarchaeologists are interested in the complex interactions between human biology, environment, culture, and individual life histories. Information gathered from the examination of human skeletal remains not only aids bioarchaeologists in the creation of individual osteobiographical profiles but provides broader information about the culture and time period in which a person lived. When teeth contain a wealth of information about an individual's life experience and relationship to their larger community.

Unlike bone, tooth enamel does not remodel once formed, making it an ideal material for bioarchaeological analysis because it forms incrementally *in utero* and over the first few years of an individual's life. Therefore, enamel provides a long view of an individual's health during the period of growth and development, from approximately fourteen weeks *in utero* to early adolescence (Hillson 1996; Hillson and Bond 1997). Emerging research, including the Developmental Origins of Heath and Disease hypothesis (DOHaD), suggest that events that happen during this period have the potential to impact long-term adult health outcomes and individual morbidity and mortality (Armelagos et al. 2009). Therefore, the study of stress and its relationship to childhood development is a critical aspect of bioarchaeological reconstructions of past populations.

Bioarchaeological research on human dentition traditionally focuses on a number of different but inter-related areas of research. These include the examination of dental morphology and metrics, which can be used to evaluate the impact of chronic stress on development (e.g.: Barrett 2012) and the assessment of biological distance between populations (e.g.: Thompson et al. 2015). Cultural practices which alter the appearance of

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teeth (e.g.: cultural modification) and surrounding bone and soft tissue in the oral cavity can also be observed (Langsjoen 1996). Teeth are also frequently used for dietary reconstruction through the examination of dental wear patterns (e.g.: Elazay et al. 1997; Tribett and Tung 2010) and dental chemistry (e.g.: Brickley et al. 2017). Finally, teeth can be used to reconstruct health during early childhood through the analysis of tooth enamel and enamel hypoplastic defects (e.g.: Guatelli-Steinberg et al. 2004; King et al. 2005; Tomczyk et al. 2012).

This thesis focuses on the analysis of linear enamel hypoplastic defects, (often abbreviated LEH), which represent interruptions in regular enamel deposition during tooth development. These defects are indicative of systemic metabolic disruption and therefore provide bioarchaeologists with an overall picture of childhood "stress events" (illness, malnutrition etc.) that occur during odontogenesis (tooth development). Using known rates of human enamel formation, the bioarchaeologists can infer the duration, frequency, and timing (age) of these events (Goodman and Rose 1990; Reid and Dean 2006; Hassett 2012; 2014).

Rinconada Alta is a coastal site located in the Rímac River Valley (Lima, Peru) between the modern-day cities of Lima and Callao (Salter-Pedersen 2011). The Yschma culture occupied Rinconada Alta during the Late Intermediate Period (AD 900-1470), and was comprised of several independently governed polities, with a shared ceremonial and religious center at Pachacamac (Shimada 2000; Owens and Eeckhout 2015). The Yschma were assimilated into the Inca Empire around AD 1470 (Lumbreras 1974). The skeletons from Rinconada Alta examined for this thesis research predominantly date to the Late Horizon after Inca conquest, but include some burials from the earlier Late Intermediate period (Diaz 2011). The site of Rinconada Alta therefore represents a unique opportunity to examine a community which experienced significant political upheaval and social reorganization due to Inca conquest and assimilation. Prior bioarchaeological studies suggest the population at Rinconada Alta remained local throughout this transition (Marsteller et al. 2017), providing a baseline for understanding how Inca conquest impacted coastal communities outside of larger centers of trade and activity like Pachacamac and Puruchuco-Huaquarones on the Central coast.

The main goal of this thesis, therefore, is to refine the analysis of LEH at Rinconada Alta as previous dental studies from the Andean region have not typically employed microscopic methods of observation (e.g.: Klaus and Tam 2009). The analysis of LEH from adult and adolescent skeletons from Inca (AD 1470-1532) and Late Intermediate periods (AD 900-1470) at Rinconada Alta broadens bioarcheologists' understanding of childhood stress on the Central coast and can be compared to Late Horizon (AD 1470-1532) and preceding Late Intermediate Period (AD 900-1470) sites throughout the Andean region. Additionally, this thesis provides stress duration estimates on a sub-sample from Rinconada Alta and compares these to stress episodes from a Late Intermediate period site on the southern coast of Peru (AD 900-1350). This comparison contributes broadly to the field of dental anthropology as there is a lack of published data on enamel defect duration from the Andean region at present. Finally, this thesis attempts to situate the site of Rinconada Alta within the larger narrative of Andean prehistory and compares the prevalence of oral pathology and average stature with two other temporally similar archaeological sites from coastal Peru.

Prior bioarchaeological research in the Peruvian and wider Andean region has included the visual analysis of LEH (Murphy 2004; Klaus 2008; Salter-Pedersen 2011), but few published studies have employed microscopic methods to examine dental defects among these populations. Microscopic, photogrammetric methods of analysis are employed broadly in dental anthropology literature from other geographic regions (e.g.: Guatelli-Steinberg et al. 2004; Temple et al. 2013; Berbesque and Hoover 2018) and provide a more accurate picture of the overall enamel surface by detecting "microdefects", which are unobservable to the naked eye (Hassett 2014). This standard should therefore be applied to in-depth dental analyses of archaeological populations from the Andean region.

This research follows the "biocultural approach" (Martin et al. 2013), which integrates aspects of biology and culture in its interpretation of health in archaeological populations. Additionally, it examines population health and its relationship to the "bioarchaeology of imperialism", which describes the social and individual impact of conquest, violence, and assimilation in archaeological populations (Tung 2012). The Inca employed a variety of military and political strategies to expand their Empire, the result of which led to differential social outcomes for populations across the Empire (Haun and Cock 2010). Therefore, this research seeks to situate the Yschma people at Rinconada Alta within the larger narrative of Andean prehistory on the Central coast of Peru.

Finally, because LEH analysis can be conducted using a variety of different methods (Hassett 2012; 2014), this MA thesis aims to clearly outline the methodology and materials used to collect dental data for the purposes of bioarcheological research. Scanning Electron Microscopy (SEM) increases the visibility of smaller surface defects, and the images obtained through SEM analysis can then be measured to estimate timing and duration of individual stress-events. The analysis portion of this MA thesis will therefore examine potential differences in rate of metabolic disruption and dental health between adult males, females, and adolescents of unknown sex at Rinconada Alta, in addition to comparing the sample as a whole to published data from the nearby site of Puruchuco-Huaquerones. It will also touch on the "Weaning Hypothesis" (e.g. Ritzman et al. 2008) in relation to the timing of linear enamel defects during early childhood. Finally, the results of LEH analysis from the Rinconada Alta sample will be compared to a Late Intermediate period sample from the Southern coast of Peru (Buikstra 1995; Knudson et al. 2007) that was examined using the same methods employed for this MA thesis.

The second chapter presented in this thesis will situate the site of Rinconada Alta within the larger framework of Andean history and cultural development. Chapter Three will provide an overview of the human physiological stress response, dental anatomy, and development (odontogenesis). It will also include a discussion of the definition of "health" and "stress" in archaeological populations, and emerging research on early childhood stress and its impact on health outcomes later in life. The Materials and Methods section (Chapter 4) of this thesis will present the data collection methods used in the field, and the specific microscopic and casting methods used to examine the teeth collected from the Rinconada Alta sample. The Results (Chapter 5) section will present analysis for frequency and duration of stress events at Rinconada Alta, compare dental health and LEH between Rinconada Alta and Puruchuco-Huaquerones, and compare length of metabolic disruption between the Rinconada Alta sample and the Late

Intermediate Period (AD 900-1350) site of Chiribaya Baja (Buikstra 1995) on the Southern coast of Peru. Chapter 5 also includes comparison of average stature in the current Rinconada Alta sample with the other two comparative, coastal Andean sites examined in this thesis. discussion and conclusion sections. The discussion and conclusion (Chapters 6 and 7) will review methodological approaches to analyzing enamel hypoplastic defects, discuss critiques of LEH analysis in relation to the "Weaning Hypothesis", and attempt to contextualize childhood stress at Rinconada Alta within the larger narrative of Andean prehistory by comparing the results of this thesis to other published data from geographically and temporally similar sites from the central and Southern coast..

The Andean region is underrepresented in bioarchaeological investigations of linear enamel hypoplastic defects using microscopic methods of analysis. This MA thesis contributes to the dental anthropology literature in this geographic region and demonstrates the potential of SEM to refine the interpretations of prehistoric stress during the critical period of childhood growth and development. Additionally, the data presented here can be compared to emerging research at other sites throughout the Andean region and can provide a baseline for understanding stress in coastal communities pre-and-post Inca conquest. While this master's thesis cannot provide an exhaustive review of methodology in dental anthropology, it attempts to partially fill a current gap in Andean bioarcheological literature and highlights the importance of expanding dental studies on archaeological populations from this region of the world. In past and present societies, the impact of social and political change on the people within these societies is rarely straightforward. It is therefore imperative that bioarchaeologists attempt to reconstruct these relationships through the remains of the individuals who lived through these experiences. Dental analysis of the human remains found at the site of Rinconada Alta provides one means of revealing information about childhood stress at Rinconada Alta from the Late Intermediate Period and the Late Horizon on the Central coast of Peru.

### CHAPTER TWO: BACKGROUND AND SITE CONTEXT

### 2.1 Introduction and discussion of previous research

The goal of this thesis chapter is to provide a general overview of the timeline of Central coast occupation, with a specific focus on sites in the Rímac and Lurín river valleys. A third site from southern Peru, Chiribaya Baja, is also discussed. Additionally, it will discuss this research in relation to the "Bioarchaeology of Imperialism" (Tung 2012) within the specific context of Inca imperialism and political control. Finally, this chapter reviews previously published research on linear hypoplastic enamel defects from the Peruvian region. The literature on the Weaning Hypothesis will be reviewed in Chapter 3 with the discussion of dental development.

This Master's thesis aims to investigate childhood stress in a sample of skeletons from Rinconada Alta and to compare these results to other coastal sites in the region. It also aims to demonstrate the usefulness of microscopic LEH analysis in populations from the Andean region. Prior to the Inca occupation of the Central coast, Rinconada Alta was inhabited by members of the cultural group known as the Yschma (alternative spelling: Ichma), which occupied the Rímac and Lurín river valleys around present-day Lima after the collapse of the Wari Empire (Llanos and Shimada 2010). There have been numerous studies conducted at the nearby sites of Pachacamac and Puruchuco-Huaquerones, which are associated with Yschma-period occupations (Eeckhout and Owens 2008; Williams and Murphy 2013), but fewer studies have been conducted at Rinconada Alta.

Populations living on the Central coast of Peru were incorporated into the Inca Empire around AD 1470 (Eeckhout and Owens 2008). The Inca were known to employ a variety of strategies for conquest, which included violent, military control (Kosiba 2010). However, the Inca frequently used existing political systems to their advantage, appointing pre-existing local elites to governing positions within the Empire (Kosiba 2010). Additionally, at significant sites throughout the Empire, the Inca resettled workers from a variety of geographic and ethnic backgrounds (Andrushko et al. 2006). There is also some evidence that the Inca incorporated aspects of local religions into their own cosmology, particularly on the Central coast (Shimada 2000). Therefore, Inca imperialism not only took the form of military-led conquest, but also included "peaceful" incorporation, population resettlement, and ideological assimilation.

To assess childhood stress at Rinconada Alta, a sample of predominantly Inca period individuals at the site will be examined for dental indicators of stress using Scanning Electron Microscopy (SEM). The timing, frequency, and duration of these enamel hypoplastic defects will be compared to provide an overall view of childhood stress-events between these two populations. A previous doctoral dissertation by Salter-Pedersen (2011) compared visual counts of hypoplastic defects between Inca-period individuals at Rinconada Alta and those from the neighboring site of Puruchuco-Huaquerones; concluding that individuals at Rinconada Alta were under more stress than those at Puruchuco-Huaquerones. However, as visual analysis can significantly underestimate the presence of fainter LEH lesions (Hassett 2012; 2014; Lacerte et al. 2016), it is worth revisiting this hypothesis.

Additionally, there appears to be some temporal confusion between the Salter-Pedersen (2011) and Marsteller (2015) dissertations on Rinconada Alta. Salter-Pedersen (2011) claims to examine health in an Yschma-period (AD 900-1470) sample, while Marsteller (2016) purports to be using a sample is from the later Inca period (AD 14701532). However, there is overlap in the two samples in both individual funeral context number (*codigo* or *context funerario*) and the sector of the cemetery from which the skeletons were recovered. For example, the same skeletons from several sectors (particularly Sector IIAE) appear in the datasets for both Salter-Pedersen (2011) and Marsteller (2015) (e.g.: RA 1058, RA 1135-1B, RA 1143, RA 1340), but each author claims they date to a different time period. Therefore, it appears that the Rinconada Alta sample used by Salter-Pedersen (2011) and Marsteller (2016) may actually be a mix of Yschma from the Late Intermediate Period and remains dating to the Inca occupation of the site.

The original field notes presented by Diaz and Guerrero (1997, 1998, 2002) suggest that while some Late Intermediate Period (AD 900-1400) burials are present at the site, along with material from the earlier Lima culture, the majority of excavated sectors at the site have layers indicating Inca-period occupation (Diaz and Guerrero 1996; Ruiz and Guerrero 1996). Much of this chronology is based on the analysis of stratigraphy, pottery styles, and grave goods recovered from the site (Diaz 2011). The sample for this thesis is drawn primarily from Sector II, subsector AE (see Appendix A) which corresponds to the beginning of the Late Yschma (Inca period) occupation at Rinconada Alta (AD 1470-1532), when the Yschma polity was incorporated into the Inca Empire. Additional individuals (see Appendix A) come from Sectors I and IV, which definitively date to the period of the Inca occupation at Rinconada Alta (Ruiz and Guerrero 1996; Diaz and Guerrero 1998). A breakdown of the cemetery sectors used in the present study is provided in the Materials and Methods section (Chapter 4) of this thesis. Despite the issues in chronology at the site, it is worth noting that the remains at

Rinconada Alta have been identified as ethnically Yschma regardless of whether they date to before or after Inca conquest (Diaz 2011, Salter-Pedersen 2011; Marsteller 2016).

Salter-Pedersen's (2011) dissertation includes a visual analysis of LEH. Given her assumption about site chronology, Salter-Pedersen (2011) uses the analysis of biological distance and skeletal pathology/stress to measure changes in stress between the assumed "Late Horizon" period of occupation at Rinconada Alta and two other Inca period sites on the Central coast, Puruchuco-Huaquerones and Chiribaya Baja. The results of Salter-Pedersen's (2011) LEH analysis should be re-evaluated as the majority of her sample has mixed chronology, rather than dating exclusively from a post-conquest sample, as was originally asserted in her dissertation.

It should be noted that with the exception of a conference presentation on timing and duration of linear enamel defects at Chiribaya Baja (Barrett and Mast 2016), there is no previously published comparative dental studies from the Andean region that use SEM or other microscopic analysis to determine stress episode duration. Therefore, this MA thesis research represents a novel application of a well-known technique to better understand childhood stress during this transitional period on the Central coast of Peru.

### 2.2 Central Coast Timeline

The Andean region, referred to by the Inca as the "four corners" (*Tawantinsuyu*), encompasses a geographically diverse region along the Western coast of South America (Moseley 2001). With the Pacific Ocean and Andean mountain range on either side, this region is host to a unique set of ecological zones, from coastal river valleys, semi-arid highlands, and tropical rainforest (Figure 2.1). The populations that occupied this region from the Formative period (1800 BC) onward adopted a wide variety of subsistence



*Figure 2.1.* Graphic representation of the different ecological zones found throughout the Central Andean region, illustrated by J. Lacerte (modified from Sandweiss and Richardson 2008).

strategies to adapt to this sometimes-hostile environment (Moseley 2001). At its height, Inca territory covered a region along the Andean coast that stretched from northern Chile to the southern region of Columbia (Moseley 2001) and was the largest empire in the New World.

The Southern Oscillation is a pattern of fluctuating ocean temperatures and currents along the equatorial Pacific coast of South America, which affects annual rainfall in the Andean region (Philander 1999). Corresponding El Niño and La Niña weather patterns can cause catastrophic flooding and trigger severe drought by disruptions to the normal Southern Oscillation cycle (Philander 1999; Satterlee et al. 2000). Therefore, irrigation and water-management were crucial to the success of ancient Andean cultures (Lamadrid 2014), with all coastal societies settling along river-valleys (Sandweiss and Richardson 2008). These settlements were often located in strategic areas, allowing particular groups control over water, irrigation systems, and arable land (Shimada 1994; Billman 2002). A general timeline of the occupation of the Peruvian Andean region is presented in Figure 2.2. Populations along what is now the Central coast of modern-day Peru generally followed these settlement patterns, with a need for resource management giving rise to the development of centralized states (polities) in the region. Human occupation on the Central coast began around 5000 BC (see Figure 2.2), with the introduction of pottery occurring after ~1750 BC in the Lurín and Rímac River valleys (Patterson and Moseley 1968). On the North coast of Peru, Billman (2002) suggests that control of irrigation and water resources, coupled with wealth from abundant marine resources may have given rise to early cultures like the Moche (200-600 BC), whose span of influence extended into the Central coast. Prior to the Inca conquest of the Central coast around AD 1470, populations living on the Central coast, including the Yschma, generally followed this settlement pattern (Rostworowski 2002). The following section provides an overview of pre-Columbian occupation on the Central coast of Peru, with a specific focus on sites located within the Rímac (Rinconada Alta, Puruchuco-Huaquerones) and Lurín (Pachacamac) drainages. Culture and environment in the Andean region are intrinsically



*Figure 2.2.* General overview of Peruvian prehistory prior to arrival of the Spanish in the Americas. Graphic created by J. Lacerte based on seriation of Andean occupation presented by Mosely (2005). Major cultural groups from the highlands and central, southern, and Northern coasts are represented. The labels for each culture are placed approximately rather than at specific start and end dates, to illustrate the cultural overlap and transition between significant cultural groups. Tick marks along the line indicate specific estimated start dates for cultural periods/horizons throughout Andean prehistory (following Mosely 2001, p. 22, 23).

linked, and therefore the geography of the land played a major role in the structure of prehistoric societies in the region.

*Lomas* refer to hilly areas of vegetation that grow during the humid Peruvian winters (Patterson and Moseley 1968). Fog from the coast provides enough moisture for vegetation to grow in these coastal desert hills, allowing human occupants to use the vegetation and wildlife in these areas as a food source, and graze camelid herds during the winter months (Patterson and Moseley 1968). This unique geographic feature meant that many early Pre-ceramic populations on the Central coast were likely semi-nomadic, moving between the coast and inland regions during different times of the year (Patterson and Moseley 1968). Patterson and Moseley (1968) note that during the summer, these earliest Peruvian occupants of the Central coast used marine resources to supplement their diet. A shift to focusing on marine exploitation likely gave rise to some of the first permanent settlements on the coast (Patterson and Moseley 1968). Emerging research at the site of El Pacifico (2200-1800 BC) between the Chancay and Lurín river valleys on the Central coast suggests early populations in the region chose centralized locations for their sites based on resource abundance/access or places with religious or symbolic importance (Flores 2017).

Peruvian ethnohistorian María Rostworowski de Diez Canseco (1915-2016) proposed that the settlement and subsistence pattern on the coast of Peru could be described using a *horizontal* model of occupation. Her account was largely based on ethnohistoric accounts of Inca conquest, social organization, and rule. This model suggests that coastal populations were grouped into *señoríos* (polities), overseen by an independent group of elites (Rostworowski 1989; 2002). Occupants living on the coast were *pescadores* (fishermen) and relied on marine resources; those that lived farther into the coastal river valleys were *labradores* (agriculturalists), who grew a variety of plants (maize, cotton) in addition to camelid herds as their primary means subsistence (Rostworowski 1989; 2005).

Señorios often formed alliances to create self-sustaining governments, frequently sharing a similar cultural style and set of religious ideologies (Rostworowski 1989). While some authors have questioned the application of this model to all settlements along the Peruvian coast (Zaro 2007), isotopic analysis ( $\delta^{13}C_{col[VPDB]}, \delta^{15}N_{col[AIR]}, \delta^{13}C_{ap[VPDB]}$ ) from the site of Rinconada Alta suggests Yschma individuals at the site generally followed this pattern of occupation and intra-group trade (Marsteller 2015; Marsteller et al. 2016).

The earliest, settled occupation on the Central coast of Peru is associated with the Lima Culture (Earle 1972), which occupied the Lurín and Rímac river valleys during the Early Intermediate Period (200 BC-AD 800). The occupation of this region by the Lima culture is contemporaneous with the Moche cultural occupation on the Northern coast of Peru, and the rise of the Nasca culture to the south (Earle 1972; Llanos and Shimada 2010). The sites of Cajamarquilla and Pachacamac are associated with both the Lima cultural occupation and subsequent Yschma culture (Llanos and Shimada 2010). It has been suggested that abandonment of Lima cultural sites in this region corresponded to flooding that occurred during the early Middle Horizon period (Satterlee et al. 2000; Llanos and Shimada 2010; Owens and Eeckhout 2015).

The site of Huaca Pucllana (located in modern-day Lima), was occupied successively by the Lima, Wari, and Yschma cultures. DNA analysis of human remains

from this site suggests a genetic link to prehistoric highland populations that continued throughout occupation at Huaca Pucllana (Valverde et al. 2016). Valverde et al. (2016) note that the serial occupation of Huaca Pucllana is largely unique when compared to other coastal sites, and genetic continuity may differ at other sites.

The emergence of the highland Wari culture marks the beginning of Middle Horizon (AD 600-1000), which had a span of cultural influence that that reached from the Moquegua valley in southern Peru to the Northern coast of Ecuador (Tribbett and Tung 2010). The collapse of the Wari state around AD 1000, coupled with severe drought around AD 1100, left the Andean region in a period of instability, where various cultural groups vied for political and geographic control (Moseley 2001; Tribbett and Tung 2010). Investigations into the influence of the Wari culture on the Central coast suggest the presence of some Wari ceramics and cultural influence on burial style (Llanos and Shimada 2010). Despite this, it does not appear that the Wari entirely replaced indigenous populations living on the coast, at least at some sites (Valverde et al. 2016). Rather, Llanos and Shimada (2010) suggest limited Wari political influence on the Central coast, with local coastal polities interacting with the Wari through trade, shared religious ideology, or in a few instances, direct conquest and resettlement.

The Middle Yschma culture occupied the Rímac and Lurín river valleys during the Late Intermediate period. Along the Chimú-influenced Chancay culture to the north, the Yschma culture occupied Peru's Central coast during a period of increased resource scarcity and competition between *señoríos* (Moseley 2001). The Yschma culture is generally considered to be a group of semi-independent *curacazgos*, or communities with an individual leader (Rostworowski 2002; Rostworowski 2005). These communities likely shared a ceremonial and religious center at the site of Pachacamac (Eeckhout and Owens 2008; Owens and Eeckhout 2015). Marsteller (2015) suggests that the Yschma were a diverse group with a mixture of local and non-local residents, variable residential mobility practices during life, and a variety of mortuary practices.

Previous research has suggested that prior to the Inca conquest, the polities of the central highlands were largely unorganized, with frequent infighting (D'Altroy 1987). An examination of the highland Wanka culture suggests low-density settlements spread throughout the region, with no clear centralized form of government (D'Altroy 1987). On the Central coast, the site of Pachacamac represents the largest Yschma settlement (Shimada 2000), which was intermittently occupied by the Lima, Wari, and Yschma cultures and continued to play a role on the Central coast after Inca conquest of the region (Shimada 2000; Llanos and Shimada 2010; Takigami et al. 2014)

### 2.3 Relevant Archaeological Sites

The site of Rinconada Alta, located in the modern-day La Molina district, Lima, Peru is the focus of this MA thesis research (Figure 2.3). It is located within the Rímac river valley in central, coastal Peru (Salter-Pedersen 2011). Bioarchaeological remains at Rinconada Alta were excavated in the 1990s and early 2000s (Salter-Pedersen 2011), and are housed at the *Museo Nacional De Arqueología, Antropología e Historia del Perú* in the Pueblo Libre district of Lima, Peru.

The history of excavations and human activity at Rinconada Alta contributed to the chronological confusion and destruction of archaeological materials at the site. Originally excavated in 1959 by José Casafranco, 120 burials were recovered in the mid-1990s which correspond to Sectors II (including the IIAE extension) and IV later identified by Diaz and Gurrerro (1998). This material includes individuals from the Lima culture as well as Inca period remains. There were several other excavations and recovery of remains between 1970-1980 when the site was used primarily for commercial sand mining. Excavations in the mid-1990s identified six independent cemetery sectors (Diaz 2011), but incomplete data and the intrusion of a modern cemetery component has contributed to confusion over the site chronology.

The Ychsma culture occupied Rinconada Alta and other contemporary coastal sites during the Late Intermediate Period (AD 900-1470), and it was assimilated into the Inca Empire along with other Central coastal sites around AD 1470 (Lumbreras 1974). An Inca cemetery site represents the largest burial component at Rinconada Alta (Diaz 2011). It had been argued previously that Rinconada Alta was likely a designated artisan community and craft production center (Diaz Arriola 2015) partly due to its proximity to



*Figure 2.3.* Google Earth Image (2019) of Rinconada Alta and surrounding sites, created by Jessica Lacerte (modified from Marsteller et al. 2016, p. 302)

the administrative site of Puruchuco-Huaquerones. However, both Diaz (2011) and Marsteller (2015) note that there is little direct evidence for this claim.

Prior research suggests that the Yschma culture was genetically similar to the earlier Lima culture, which occupied the Rímac river valley during the Early Intermediate period (Valverde et al. 2016). After Inca conquest, it appears that the inhabitants of Rinconada Alta were not resettled from other parts of the empire, unlike the inhabitants of the neighboring site of Pachacamac (Salter-Pedersen 2011; Bethard 2013).

Earlier research on adult diet and health at Rinconada Alta and the nearby administrative center of Puruchuco-Huaquerones (P-H) seems to indicate that individuals at Rinconada Alta (RA) experienced slightly increased levels of stress relative to Puruchuco-Huaquerones (Salter-Pedersen 2011). Stable isotopic analysis performed by Marsteller et al. (2016) suggests that during the Middle Yschma (Late Intermediate Period) period (see Fig. 2.2), the occupants of Rinconada Alta were primarily agriculturalists with relatively little marine intake, despite their relative proximity to the coast. Dietary analysis from the nearby site of Armatambo (which is located directly on the coast) suggests that the population there consumed higher levels of marine resources than RA, but their diet also included some agricultural products (Marsteller et al. 2016). These results are consistent with the *horizonality* model put forward by Rostworowski (1989; 2005), as these populations appear to have had somewhat specialized diets and subsistence strategies while still trading amongst themselves. Slight deviations in diet at both sites suggest the populations at Rinconada Alta and Armatambo engaged in some degree of trade and had local access to both marine and agricultural products to supplement their diets (Marsteller et al. 2016).

The site of Pachacamac is located in the Lurín river valley, a half-kilometer from the Pacific Ocean (Eeckhout and Owens 2008). The site is named after the Yschma deity *Pacha Kamaq*, a creator god later adopted into the Inca religious pantheon (Llanos and Shimada 2000). Pachacamac is characterized by monumental architecture beginning with the Lima Period "Old Temple" (Eeckhout and Owens 2008). During the Middle Horizon, Wari ideological influence can be observed at the site, in addition to the rise of an oracle cult (Eeckhout and Owens 2008). A new temple, the "Painted Temple" was built during the Middle Horizon period (AD 600-1000), which likely helped facilitate the expansion of Wari religion and influence throughout the region (Eeckhout and Owens 2008). With the collapse of the Wari Empire around AD 1000, the site's influence in the coastal region declined during the Yschma occupation, until the Inca incorporated the region around AD 1470 (Eeckhout and Owens 2008; Marsteller et al. 2016).

During the Inca occupation of the Central coast, a Sun Temple, painted temple, and an *Acllahuasi*, which housed the Sapa Inca's "chosen women", were constructed at the site (Eeckhout and Owens 2008; López-Hurtado Orjeda 2011). Additionally, the oracle at Pachacamac received renewed attention under Inca rule, with pilgrims visiting Pachacamac from throughout the Empire (Eeckhout and Owens 2008). Bioarchaeological research at the site of Pachacamac suggests that some human sacrifice took place there; most of the victims appear to be adult males and infants (Eeckhout and Owens 2008).

Biological distance analysis at this site suggests that during the Inca period, individuals at Pachacamac were relocated or resettled from other parts of the Andean region (Bethard 2013); the latter being a tactic often employed by the Inca to control the population of their vast empire (Haun and Cock 2010). This does not appear to be the case at the nearby sites of Rinconada Alta and Puruchuco-Huaquerones, two Yschma sites that were also under Inca control (Williams and Murphy 2013; Marsteller 2015). Isotopic data from Rinconada Alta presented by Marsteller (2015) supports this. Despite the mixed chronology, individuals from both periods at Rinconada Alta appear to be local and not resettled from other parts of the empire (Marsteller 2015; Marsteller et al. 2016).

The increased presence of non-local individuals at Pachacamac is consistent with the renewed religious significance at the site during the Inca period and could suggest voluntary relocation to the area, rather than Inca-enforced resettlement (Bethard 2013). López-Hurtado Orjeda (2011) suggests that although religion and culture from the Central coast influenced the Inca pantheon, the Inca did alter the site to conform to their own religious and cultural ideology. For example, the Inca constructed a sun temple at the site (López-Hurtado Orjeda 2011).

These examples suggest that Inca imperial tactics varied by site, rather than employing a single method of imperial control over the entire region of the Central coast (Bethard 2013). Overall, Inca conquest on the Central coast appears to have been less violent than in other parts of the empire, particularly at non-Inca sites in the highland region (Andrushko and Torres 2011). However, individuals at the nearby site of Puruchuco-Huaquerones exhibit injuries that are consistent with Inca-style weapons, suggesting some violent conflict did occur in the area (Murphy 2004).

The site of Puruchuco-Huaquerones is located in the Rímac river valley; the site contains several cemetery sites as well as the modern settlement of Túpac Amaru (Williams and Murphy 2013). Today, many archaeological sites in the city of Lima and

the surrounding region are threatened by the encroachment of modern-day population expansion, and Puruchuco-Huaquerones is a prime example of this prehistoric/modern overlap. The site was occupied intermittently during the Initial period (1800-900 BC), Early Intermediate period (200 BC-AD 600), and Late Horizon (AD 1476-1532) (Cock and Goycochea 2004; Williams and Murphy 2013). Puruchuco-Huaquerones is significant because it is one of the largest cemetery sites in the Andean region, containing between 220 and 2400 well-preserved individuals from over one thousand burials excavated from cemeteries throughout the site (Williams 2005; Williams and Murphy 2013).

Several *khipu* (knotted cords) recovered from the site have led researchers to suggest Puruchuco-Huaquerones was an Inca administrative center during the Late Horizon occupation (Murphy 2004; Cock and Goycochea 2004; Gaither and Murphy 2012). Due to the large number of mummified and skeletal individuals recovered from this site, a number of bioarchaeological studies and doctoral dissertations have examined the impact of Inca and Spanish conquest at Puruchuco-Huaquerones (Murphy 2004; Williams 2005; Gaither and Murphy 2012; Williams and Murphy 2013).

Isotopic analysis conducted by Williams (2005) as part of her doctoral dissertation work suggests that individuals at Puruchuco-Huaquerones were local and were not resettled from other parts of the Inca Empire. Additionally, Williams (2005) found that marine resources during this period contributed surprisingly little to the diet of the occupants at the site. Some of the dietary and dental health differences observed between males and females are likely related to female production of *chicha* (corn beer), which was produced by fermenting plants like maize or quinoa (Williams 2005; Williams

and Murphy 2013). However, after Inca conquest at the site, chicha consumption likely decreased, as more resources were likely spent producing food that would be distributed throughout the empire (Williams and Murphy 2013).

Overall, it appears that Puruchuco-Huaquerones acted as an administrative center after the Inca conquest, and was largely inhabited by local, coastal populations (Williams 2005; Williams and Murphy 2013). The examination of non-specific stress indicators among individuals at the site suggests the occupants of Puruchuco-Huaquerones experienced some periods of poor health and nutrition, but many survived these events and showed signs of healing (Williams and Murphy 2013). Williams and Murphy (2013) conclude that Inca occupation of Puruchuco-Huaquerones did not severely impact health



*Figure 2.4.* Google Earth Map of the Osmore Drainage (2019) by Jessica Lacerte. Includes primary Chiribaya-affiliated cemetery sites (modified from Buikstra 1995, p. 231).

at the site, but food shortages brought about by the Inca labor demands could have contributed to periods of malnutrition at the site. Chiribaya Baja is a Southern coastal site located in the Osmore drainage, a river valley approximately 870 km south of Lima (Figure 2.4). Chiribaya Baja is one of seven sites in this valley associated with the Chiribaya culture, a group that occupied southern Peru and parts of northern Chile during the Late Intermediate period (900-1300 AD). This culture practiced both natural and artificial mummification (Guillen 2004) and is known for the distinct white-dotted rims on their pottery. This site was chosen for comparison with Rinconada Alta because Chiribaya Baja is the only other Andean sample with published results of LEH observation using Scanning Electron Microscopy instead of visual recording methods (Barrett and Mast 2016). This allows us to directly compare the duration of stress events during childhood with those at Rinconada Alta.

Chiribaya Baja is located 8 km from the Pacific coast and is surrounded by the remains of irrigation systems and residential and agricultural terraces (Buikstra et al. 2001). The occupation of Chiribaya Baja dates to approximately AD 1025 (Martinson et al. 2003). Two Chiribaya cemeteries associated with this site were excavated in 1991 (Buikstra 1995). Although Chiribaya Baja is located relatively close to the Pacific Ocean, there is a lack of fishing implements and other marine-related objects in burials from this site (Tomczak 2003). Paleodietary studies, the presence of grave goods associated with *labradores* (agriculturalists), and the remains of agricultural infrastructure suggest that maize cultivation was the primary means of subsistence at Chiribaya Baja (Tomczak 2003; Zaro 2007; Knudson et al. 2007).

The author of this thesis spent two field seasons during May-June 2015 and 2016 at the *Centro Mallqui Instituto de Bioarchaeología* located in El Algarrobal, Peru collecting data as part of a bioarchaeology field school at Washtenaw Community College located in Ann Arbor, MI. Dental casts collected from these field seasons were analyzed by Barrett and Mast (2016) using Scanning Electron Microscopy and presented at the 2016 BARFAA conference in Chicago, Illinois. At the time of this thesis, this is the only other sample from the Pre-Columbian Andean region that has used microscopic techniques to examine duration of stress events in an archaeological sample from Peru.

### 2.4 Bioarchaeology of Imperialism

The variable role of sites on the Central coast during the period of Inca occupation raises several questions of bioarchaeological interest. Previous research (Salter-Pedersen 2011) has attempted to examine the impact of the Inca conquest at Rinconada Alta. However, given the issues with chronology addressed at the opening of this chapter, these must be reevaluated.

The "bioarchaeology of imperialism" (Tung 2012) examines the biological and social consequences of conquest by studying the skeletons of individuals under imperial control. In the Americas, and Andean region in particular, there are several bioarcheological studies that focus on the impact of European colonialism on indigenous health (e.g.: Klaus and Tam 2009; Klaus 2010, Murphy et al. 2010). However, prior to Spanish conquest around AD 1532, several cultures throughout Andean prehistory, including the Inca, conquered neighboring populations through a variety of strategies including, but not limited to, military conquest (Bethard 2013). Therefore, the majority of publications on the impact of imperialism in the Andean region focus on militaristic cultures like the Wari and Inca prior to the arrival of the Spanish (e.g.: Andrushko et al. 2006; Tung 2012; Bethard 2013).

Strategies for conquest can involve violent, military takeover by a dominant group, but may also include "peaceful" incorporation, or ideological control through the spread of a dominant religion (Andrushko et al. 2006; Tung 2012). After conquest, the Inca frequently "resettled" individuals from multiple regions across the empire, as one of their methods for controlling conquered populations (Bethard 2013). Alternatively, preexisting local elites were sometimes incorporated into the Inca hierarchy of governance (Bethard 2013).

Families within the empire were organized into ayllus, groups of relatives who worked together to provide goods/labor related to agriculture and distribution (Malpass 1996). Endogamy was common, solidifying these familial relationships and their hold over agricultural lands (Malpass 1996). The Inca also employed *m'ita* labor tax on the *ayllu*, a system by which individuals were rotated to provide labor for the building of public works and monumental construction projects (Malpass 1996). The increased demands of this type of labor on local populations had the potential to disrupt health (Williams and Murphy 2013). Therefore, bioarchaeologists working in the Andean region are broadly interested in examining the relationships between conquest, resulting trauma, and differential resource access. The next section delves further into specific published examples of the "Bioarchaeology of Imperialism" to better understand the impact of the Inca conquest on cultural groups in the Andes, and more specifically, on the Central coast.

Populations under Inca control often experienced increased social stratification as the result of conquest (Diaz and Vallejo 2004; Bethard 2013). These changes may be reflected in the amount of non-specific stress experienced during childhood due to differential access to resources. Therefore, this thesis is interested in comparing childhood stress from Rinconada Alta to the Inca-period site of Puruchuco-Huaquerones.

While Tung (2012) originally focused on the effects of the Wari Empire's conquest throughout the Andean region, several published bioarchaeological studies and dissertations examine the impact of Inca military action and assimilation during the Late Horizon. Andrushko et al. (2006) examined burials at two highland sites, Sacsahuaman and Chokepukio in an attempt to compare health between Inca elites (Sacsahuaman) and a local population of "commoners" (Chokepukio). Andrushko et al. (2006) found that the majority of burials at the elite site of Sacsahuaman belonged to older women, while Chokepukio had a much more even distribution of males/females and adults/subadults.

Overall, joint disease and trauma were higher at Chokepukio, but rates of osteoporosis were higher in the sample from Sacsahuaman. Cranial modification was more common in the sample from Chokepukio, and individuals from the site displayed a variety of modification styles, suggesting a wider variety of ethnic groups at the site (Andrushko et al. 2006). Andrushko et al. (2006) suggest health at Chokepukio was impacted by increased labor demands, but that the Inca elites at Sacsayhuaman still experienced poor health in the form of dental and age-related joint disease.

Andrushko and Torres (2011) also examined 11 cemetery sites near the Inca capital of Cusco for evidence of warfare, comparing the bioarchaeological data to the information presented in the Spanish Chronicles. The Spanish chronicles provide a biased but invaluable window into pre-Columbian activity in the Andean region. Many accounts suggest that war with the Chanka ethnic group was the catalyst for larger Inca imperial expansion (Andrushko and Torres 2011). In an attempt to evaluate these claims, Andrushko and Torres (2011) examined skeletons from the Middle Horizon, Late Intermediate period, and Late Horizon for evidence of blunt-force cranial trauma caused by pre-Columbian Andean weapons such as clubs. Results from Andrushko and Torres (2011) suggest that cranial trauma in the Middle Horizon was almost non-existent, with a 2.8% frequency in the Late Intermediate period. In the Late Horizon, there is a jump in frequency to 7.8%. However, this prevalence is still relatively low for a group embroiled in major conflicts, leading the authors to conclude that the Inca employed peaceful negotiation more frequently than direct military force, at least in the Cusco region (Andrushko and Torres 2011).

Dissertation work by Bethard (2013) provides a comprehensive overview of Inca resettlement practices within the framework of the "bioarchaeology of imperialism". Using craniometric data to determine biological distance, Bethard (2013) examined 552 individuals from nine archaeological sites from Peru. Five Inca Late Horizon sites were examined (Huaquerones & 57AS03 (Puruchuco-Huaquarones), Pachacamac, Machu Picchu, Colmay) and four non-Inca sites (Yauyos, Cajamarca, Ancón, and Makatampu). These sites are a mix between the Central coast and sierra regions of the Peruvian Andes (Bethard 2013).

Populations at the Central coast sites of Huaquerones and 57AS03 (both located within the larger complex of Puruchuco-Huaquarones), appear mostly homogenous when compared to individuals from key Inca sites like Colmay and Machu Picchu, which represent up to six different regions of geographic ancestry (Bethard 2013). This is significant as it suggests different patterns of relocation and settlement after Inca conquest between the highlands and Central coast (Bethard 2013).
The Central coast site of Pachacamac also appears to have been resettled, likely due to the presence of the Pachacamac cult and its significant role within Inca cosmology (Eeckhout and Owens 2008; Bethard 2013). An alternative explanation for this observation is increased pilgrimage and intentional settlement at the site, rather than the more common "forced" resettlement practiced by the Inca (Eeckhout and Owens 2008). Bethard's (2013) analysis suggests that the Inca used resettlement on the Central coast selectively, and it was not necessarily the primary means of political control at sites like Puruchuco-Huaquarones and possibly Rinconada Alta.

Additional support for the use of resettlement can be seen at several Wanka sites from the highlands of Peru (D'Altroy 1987). Prior to Inca conquest, research suggests the Wanka were an ethnic group without a seat of centralized power; this lack of organization likely led to infighting among the Wanka (D'Altroy). Under Inca rule, the Wanka elites became more powerful, allowing their influence to spread throughout the region (D'Altroy 1987). This example highlights the complicated ways in which imperialism can be expressed in the bioarcheological record and suggests a similar means of controlling local elites may have been employed elsewhere in the Inca empire.

Williams and Murphy (2013) used stable isotopic analysis alongside the analysis of skeletal and dental pathological lesions to examine health at the Central coast site of Puruchuco-Huaquarones. They focused on dietary reconstruction and evidence of carious lesions and other nutrition-related pathologies to evaluate potential changes in diet as a result of Inca conquest (Williams and Murphy 2013). The authors note that hypoplastic dental lesions were not included in their publication as their onset occurs during childhood development (Williams and Murphy 2013). However, both Williams (2005) and Murphy (2004) published individual dissertations that focused on health and diet at Puruchuco-Huaquerones.

The data presented in these studies suggests that overall health did not change significantly at Puruchuco-Huaquarones after the Inca incorporation, so it is similar to other sites from the Late Horizon (Williams and Murphy 2013). However, some increased antemortem tooth loss from this period suggests some dietary shifts that impacted oral health. Since Puruchuco-Huaquarones is temporally and geographically close to Rinconada Alta, research at this site can be used for comparative purposes. However, the lack of specific data on linear hypoplastic defects highlights the need for more in-depth dental studies from pre-Columbian Peru, particularly from the Central coast region.

# 2.5 Peruvian Bioarchaeological Dental Studies

Most previous bioarchaeological dental research on populations from pre-Columbian Peru and the Andean region has focused on oral health (dental wear etc.) and the effects of regional cultural practices like coca chewing (e.g.: Elzay 1977; Indriati and Buikstra 2001). Several journal articles and dissertations focus specifically on dental health from coastal Peru (Klaus 2008; Salter-Pedersen 2011; Penzo-Lanfranco et al. 2017). The visual analysis of linear hypoplastic defects has been included in several dissertations (Farnum 2002; Salter-Pedersen 2011; Dillon 2015). A dissertation by Livengood (2012) used SEM to examine microwear on a sample of teeth from the Inca site of Machu Picchu. Barrett and Mast (2016) presented a comparison of linear enamel defects between the Peruvian Chiribaya and Point Hope Inupiat (Alaska). With these exceptions, no previous (published) research from the Andean region has used SEM to examine dental samples from Peru.

Elzay et al. (1977) examined a sample of dental casts from five several Pre-Columbian cultures from Peru: Paracas, Nazca, Tiwanaku, Ica, and Inca. The three coastal cultures (Nazca, Paracas, Ica) represented in the study had higher instances of caries than highland and *altiplano* cultures (Tiwanaku, Inca) (Elzay et al. 1977). The highland cultures also had a higher frequency of congenitally missing and un-erupted third molars than the coastal groups (Elzay et al. 1977). The Elzay et al. (1997) article represents an early bioarchaeological attempt to understand differences in oral health between cultural groups throughout the Andean region.

Penzo-Lanfranco et al. (2017) re-evaluated the argument that coastal and highland populations in pre-Columbian Peru exhibit distinct patterns of oral health/disease. Two coastal Late Intermediate period sites, Armatambo (n=25) and Los Pinos (n= 200), were selected for the study, while the highland samples were taken from Late Intermediate period (n= 55) and Inca period (n= 23) material from Laguna de los Cóndores, which is associated with the Chachapoyas culture (Penzo-Lanfranco et al. 2017). The goal was to examine the effect of carbohydrate-rich diets on populations from both time periods. Coastal populations generally consumed more maize, while highland populations focused on potatoes, quinoa, and root tubers; plants that typically grow at higher altitudes (Penzo-Lanfranco et al. 2017). This dietary difference, along with varying cultural methods of preparing and cooking these carbohydrates, contributed to the patterns of oral health observed between coastal and highland populations (Penzo-Lanfranco et al. 2017). Overall, populations on the Central coast appear to have more caries than populations from either time period at the site of Laguna de los Cóndores in the "Cloud Forest" region of northern Peru (Penzo-Lanfranco et al. 2017).



*Figure 2.5.* Example of the effects of coca quid use on oral cavity health. Note the blackened calculus on the molars and severe root exposure (Chiribaya Baja, 2015, photo credit: J. Lacerte)

The practice of coca (*Erythroxylum coca*) chewing among pre-Columbian cultures is another factor that impacts studies of oral health (Langsjoen 1996; Indriati and Buikstra 2001; Penzo-Lanfranco et al. 2017). Coca quids are placed between the cheek and the buccal side of the molars for absorption into the bloodstream via the oral mucosa (Indriati and Buikstra 2001). Quicklime was added to enhance the release of alkaloids in the leaves (Indriati and Buikstra 2001). This process creates an alkaline oral environment, which can increase the presence of distinctive dental lesions (see Figure 2.5), including: severe root exposure (particularly of the molars), caries at the cervix, and buccal surface and tooth loss when combined with other indicators of coca chewing (Langsjoen 1996; Indriati and Buikstra 2001) such as chemical analysis.

Brown (2012) examined hair samples from 11 mummified individuals from Puruchuco-Huaquerones for evidence of coca consumption. The author found that 8 of them tested positive for benzoylecgonine, one of the major compounds found in cocaine (Brown 2012). This practice was widespread throughout the Andean region and was also present on the Central coast (Langsjoen 1996; Brown 2012; Penzo-Lanfranco et al. 2017), so its effect on oral health cannot be overlooked.

*Chicha* production is another cultural factor that has the potential to impact health, particularly among women, who prepared maize (or quinoa) for fermentation by masticating it into a paste (Goldstein 2007). During the Inca period, "chosen women" known as *acllacona*, or the "Virgins of the Sun", were tasked with brewing *chicha* for ceremonial and religious festivals. Several *Acllahuasi* sites from the Inca period have been identified including one at Pachacamac; it is likely that *chicha* was also produced and consumed by a similar institution prior to Inca conquest (Eeckhout and Owens 2008; Penzo-Lanfranco et al. 2017). It is important to consider the oral health effects associated not only with *chicha* production, but also *chicha* consumption on populations from this region, as oral acidity and alkalinity have the potential to be altered by the combined effects of coca and chicha (Penzo-Lanfranco et al. 2017)

As previously discussed, a number of dental studies from the Andean region have included the analysis of visual counts of linear enamel hypoplastic defects (LEH). While visual methods of observing these defects are known to underrepresent the total number of defects per tooth (Hassett 2012; 2014), these studies still provide a baseline for understanding linear hypoplastic defects among pre-Columbian populations from Peru. As part of her doctoral dissertation, Salter-Pedersen (2011) examined LEH at the site of Rinconada Alta and Puruchuco-Huaquerones. It should be noted that Salter-Pedersen (2011) included both adolescent and adult individuals in her analysis; some previous research suggests that there is a slight positive correlation between age-at-death and the number of LEH observed per tooth (King et al. 2005; Temple 2014). Salter-Pedersen (2011) found that a relatively even percentage of males (72.2%, n= 26) and females (73.1%, n= 19) at Rinconada Alta exhibited LEH.

Salter-Pedersen (2011) concluded that individuals at Rinconada Alta had higher frequencies of LEH (76.0% of individuals) than those from the nearby site of Puruchuco-Huaquerones (52.6% of individuals; Murphy 2004). Despite this, she notes that some of this difference could be due to the difference in age distribution between the two samples or quality of life (Salter-Pedersen 2011). Overall, the frequencies of LEH from the two coastal sites were similar (63.9% of individuals) to those recorded at the Northern coastal site of Lambayeque (Klaus 2008), but much higher than the LEH counts reported for the Inca capital city of Cusco (4.6% of individuals) (Andrushko 2007; Bethard 2013). Dillon (2015) visually assessed LEH counts from skeletal remains at the sites of Huaca Gallinazo (n= 6) and Huaca Santa Clara (n= 16) on the North Coast of Peru. These skeletons used for analysis dated to two time periods, the Virú period (200 BC-AD 600) and Tomaval period (AD 750-1150). Dillon (2015) found that 37.5% of individuals examined (n= 6/16) from Huaca Gallinazo had evidence of LEH.

The examples from the sites presented above suggest that LEH frequency is somewhat variable between pre-Columbian populations from the Andean region. While the disparity in LEH frequency between the sites of Puruchuco-Huaquerones and Rinconada Alta could reflect a difference in ability to buffer stress, it is also likely that sample size, dental and skeletal preservation, as well as the severity of oral pathology play a role in a the expression of LEH in any given sample.

For example, Lowman (2017) suggests in her dissertation that Middle Horizon period individuals at the site of Tumilaca la Chimba, Peru do not exhibit any LEH. This site is associated with two occupations, the first during the terminal Middle Horizon (AD 950-1250) and the second during the Late Intermediate Period (AD 1250-1476) (Lowman 2017). Lowman (2017) argues that individuals from the earlier settlement do not appear to have any LEH, while four of the 23 individuals (17.3%) from the LIP phase of occupation have possible linear hypoplastic defects (Lowman 2017).

However, the presence of *cribra orbitalia* and porotic hyperostosis in the Middle Horizon sample calls this assumption about stress into question if these two indicators of stress potentially share an underlying etiology (Lowman 2017). Lowman (2017) attempts to explain this by suggesting that the cause of the porotic lesions observed in skeletons from this period is indicative of a condition that affected vitamin deficiency, but not overall growth (i.e.: parasitic infection). Parasitic infection has been experimentally linked to enamel hypoplastic lesions in sheep (Guatelli-Steinberg and Lukacs 1999) and is associated with LEH presence in *Pan paniscus* (Tsukamoto 2003), suggesting that at least in some cases, parasitic load can cause metabolic disruption (and subsequent linear hypoplastic lesions) in mammals.

The images of the mandibular and maxillary teeth included in the appendix of Lowman's (2017) dissertation provide a possible explanation for the lack of LEH. There is clear, extensive postmortem damage and diagenesis observable in photographs of the skeletal material from both occupational periods; this suggests the lack of observable LEH from the Middle Horizon period may be largely due to poor preservation, rather than the total absence of LEH in this sample (Lowman 2017, p.83). Lowman's (2017) dissertation research is presented here to highlight some of the potential difficulties of observing LEH by eye in archaeological populations from the Andean region

## 2.6 Summary

The timeline of human occupation on the Peruvian Central coast, including the Yschma occupation in the Rímac and Lurín river valleys, is an under-studied area of Andean research. Archaeological and bioarchaeological investigations from sites on the Central coast suggest a long and varied history of pre-Columbian occupation, beginning with Preceramic cultures. Evidence suggests that cultures that settled along the coast generally followed the horizontal model of resource exchange suggested by Rostworowski (1989; 2005), so sites could have access to both marine and agricultural resources for these populations (Marsteller et al. 2016).

It is clear that Yschma religious ideology impacted Inca religious activities on the Central coast, particularly at the Lurín valley site of Pachacamac (Llanos and Shimada 2000). The site of Rinconada Alta, which is the area of study for this Master's thesis, is of particular interest because it is reported to have a slightly different pattern of expression of non-specific indicators of stress than the nearby administrative center of Puruchuco-Huaquerones (Salter-Pedersen 2011).

Finally, it is clear that the percentage of individuals with macroscopically visible LEH varies somewhat between pre-Columbian sites in the Andean region (Andrushko 2007; Salter-Pedersen 2011; Dillon 2015). While Inca-period sites on the central/Northern coast appear to have slightly different frequencies of LEH, they are more similar when compared to LEH frequencies from the Inca heartland around Cusco, which are significantly lower (Klaus 2008; Salter-Pedersen 2011). Therefore, investigating the frequency of LEH at Rinconada Alta could provide a baseline for understanding childhood stress on the Central coast, and be used for comparison to other sites pre-and-post Inca conquest.

#### CHAPTER THREE: STRESS AND DENTAL DEVELOPMENT

### 3.1 The Physiological Stress Response

This chapter uses the "biocultural" framework (Martin et al. 2013) to examine the relationship between dental development and stress. The biocultural approach integrates human physiology (biology) and cultural behavior to aid in the interpretation of health in past populations (Goodman et al. 1988, Martin et al. 2013). Stress is defined here as an interruption to *homeostasis*, the regulation of normal bodily function by the endocrine and related bodily systems (Webb et al. 2010). Humans experience both physiological and psychological stress, meaning their physical body and mental state may be impacted as a result (Mariotti 2015). Skeletal (and dental) indicators of stress are the primary means by which bioarchaeologists interpret stress in the archaeological record.

These skeletal indicators include but are not limited to: adult stature (as a measure of growth outcome), paleopathology, skeletal trauma, and physical evidence of growth disruption in the long bones (Harris lines) and teeth (enamel hypoplastic defects, dental fluctuating asymmetry) (Goodman et al. 1988). It should be noted that some physiological responses to chronic stress are not necessarily preserved in bioarcheological contexts. In cases where soft tissue is still present, either through artificial or natural mummification, it may be possible to extract cortisol from hair samples of deceased individuals (e.g. Webb et al. 2010) and examine the internal organs for signs of heart disease and other chronic illnesses. For example, Webb et al. (2010) provide a dynamic record of stress from a suspected victim of human sacrifice in the months leading up to their death. The hypothalamic-pituitary-adrenal (HPA) axis regulates the body's response to stress, by releasing adrenocorticotrophic hormone (ACTH) which stimulates the production of the cortisol, along with other glucocorticoids in the adrenal cortex (Herman and Cullinan 1997; Herman et al. 2012). This hormonal cascade, under regular homeostasis, acts as a negative feedback loop to reduce the effects of stress on an organism (Herman et al. 2012).

When an organism experiences a stressful external stimulus, neurons in the paraventricular nucleus (PVN) release ACTH, which includes corticotropin-releasing hormone (CRH) and arginine- vasopressin (AVP), which in turn trigger the release of glucocorticoids (Herman and Cullinan 1997; Mariotti 2015). However, the body interprets different forms of stress using slightly different pathways. Some stressors (fear, psychological abuse) trigger a negative PVN response as bodily threats and other physiological stimuli are processed more directly by the PVN (Herman and Cullinan 1997).

Stress can be divided into two categories that reflect duration: *acute* (short-term) stress, and *chronic* (long-term) stress. Acute stress refers to short metabolic insults due to infection, weaning stress experienced during early childhood, and short periods of food insecurity or malnutrition (Goodman and Armelagos 1989; Klaus and Tam 2009). Examples of chronic stress include progressive infections like tuberculosis, prolonged malnutrition, parasitic infection (ex.: leishmaniasis) (Goodman and Armelagos 1989; Klaus and Tam 2009; Marsteller et al. 2011).

*Allostasis* refers to the body's hormonal response to acute or chronic stress, as it attempts to ameliorate stress and regain homeostasis (McEwan 2000). *Allostatic load*,



*Figure 3.1.* Four examples of allostatic load, with the normal stress response presented in the table on top, from McEwan (2000, p. 110). Reproduced with permission).

therefore, refers to the cumulative effect of long-term stress throughout the life of an organism (McEwan 2000). Hypertension (high blood pressure), elevated corticosteroid levels, and decreased metabolism are some examples of clinical indicators used to measure allostatic load (McEwan 2000). Prolonged, chronic exposure occurs when the body is unable to adapt to stress or when the stressor is not removed.

Figure 3.1. illustrates several examples of allostatic load, which include: repeated periods of acute stress, lack of adequate adaptation to stress, elevated hormone levels due to a prolonged stress response, and inadequate hormonal response to elevated levels of stress (McEwan 2000). The physiological and skeletal effects of repeated exposure to stress are most prominent during early growth and development, as these processes can

be delayed or interrupted as the body attempts to regain homeostasis (Cook and Buikstra 1979).

The diagram below (Figure 3.2.) illustrates the process by which external stressors trigger the body's allostatic response and create metabolic disruption. Environmental stress refers to external factors like diet and resource procurement, which place physiological strain on an individual (e.g.: malnutrition) (Goodman and Armelagos 1989). Examples of "culturally induced" stress include warfare and social inequality (Goodman and Armelagos 1989). Cultural adaptation serves as an additional "buffer"



*Figure 3.2.* Flowchart depicting effect of environment and culture on stress and population health, from Goodman and Armelagos (1989, p. 226). Reproduced with permission.

against both environmental and culturally induced stress (Goodman et al. 1988; Goodman and Armelagos 1989). However, when these systems fail, metabolic disruption occurs, having a resulting negative impact at the individual and population level. When this disruption occurs during growth and development, the resulting "skeletal indicators of stress" provide a good marker of overall population health, as they can estimate the duration and frequency of stress that occurred during childhood (Goodman and Armelagos 1989). Therefore, the period of childhood growth and development is of particular interest to bioarcheologists.

### 3.2. Health and Stress in the Bioarcheological Record

Infants and children represent a particularly vulnerable section of the human population when compared to older individuals, and they experience higher rates of morbidity and mortality in both modern and archaeological contexts; particularly in situations where normal "cultural buffers" fail to protect them (Goodman and Armelagos 1989). Goodman and Armelagos (1989) note that infants undergoing weaning are likely to experience acute periods of diarrhea, respiratory infection, and malnutrition. However, Wright and Yoder (2003) note that not all hypoplastic defects that occur within the period of crown formation may be associated with this major early-life event.

Prior to discussing the relationship between stress and dental development and lesions, it is important to consider the definition of "health", "stress", and "frailty" in archaeological populations. The definition of health in bioarcheology can differ significantly from the modern, clinical view of health (Temple and Goodman 2014). Temple and Goodman (2014) describe *health* as the complex relationship between physiological homeostasis, "cultural buffering systems" and individual genetics, susceptibility, and immune response. The World Health Organization defines health as "…a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity" (Constitution of the World Health Organization 1946).

Reitsema and McIlvaine (2014) provide a bioarchaeological definition of health as a "holistic concept" which encompasses individual physical and mental wellbeing, a person's "quality of life", and quality of interactions within their larger community. Therefore, there is a "gap" in our understanding of health in the archaeological record, as skeletal and dental indicators of stress alone do not help bioarcheologists interpret the psychological effects of stress on an individual and community wide level (Reitsema and McIlvaine 2014).

The concept of "frailty", defined as individual susceptibility to disease/death, in bioarcheology is emphasized by the Developmental Origins of Health and Disease Hypothesis (DOHaD). Alternatively known as the "Barker hypothesis" for the pioneering work of British epidemiologist David Barker (Armelagos et al. 2009), DOHaD suggests a direct link between outcomes of adult health and the influence of maternal stressors and development *in utero* (Weisensee 2013). For example, low birth weight is often associated with increased risk of death and morbidity throughout life (Montoya-Williams et al. 2017) Additional research suggests that beyond the intrauterine environment, the period of early childhood continues to play a crucial role in adult health outcomes (Kuzawa et al. 2010). Therefore, fetal exposure to maternal stress and early childhood exposure to stress can impact individual disease susceptibility and mortality throughout life (Montoya-Williams et al. 2017).

One influence of maternal stress on a developing fetus occurs through the process of DNA methylation (Montoya-Williams et al. 2017). This occurs when DNA is altered through the addition of methyl groups to cytosine, one of the four nitrogenous bases that make up the strands of DNA (Jin et al. 2011). The addition of these methyl groups alters the function of genes, which can trigger a cascade of changes, and impact later susceptibility to disease (Jin et al. 2011). For example, maternal stress can alter the genes *IGF1* and *IGF2*, which are partly responsible for human growth and development (Montoya-Williams et al. 2017). In clinical settings, there has been an association reported between mothers experiencing chronic stressors (warfare, sexual assault, and physical abuse) and resulting *IGF1* and *IGF2* methylation, and babies with lower birth weights (Montoya-Williams et al. 2017). Gene methylation has also been shown to influence the body's inflammatory response and create susceptibility to obesity later in life (Wu et al. 2018). These emerging hypotheses about disease susceptibility indicate that stress is not just experienced at the individual and community level, but can be indirectly experienced on an intergenerational level, affecting an individual's ability to "buffer" stressors throughout the course of their life, regardless of external conditions after birth (Wu et al. 2018).

The DOHaD hypothesis has been incorporated into bioarcheological research on health (Armelagos et al. 2009), with attention being paid to skeletal indicators of stress, since many of the chronic conditions examined in modern, clinical research cannot be evaluated using skeletal remains alone (Armelagos et al. 2009). In bioarcheology, the relationship between "health" and skeletal indicators of stress is not always a direct one. Skeletal trauma (accidental or intentional bodily injury), infection, and disease can leave direct pathological lesions on bone; the appearance of which can provide information about disease etiology, progression, and potential recovery. Histological and DNA investigations of bony lesions can help bioarcheologists to accurately determine the specific cause of the lesion (Wright and Yoder 2003). However, enamel hypoplastic defects, dental fluctuating asymmetry, Harris lines, and adult stature are *non-specific* stress indicators, meaning they do not have a directly discernable cause, and are often the result of cumulative, prolonged, chronic stress on an organism, or repeated bouts of acute illness or malnutrition (Wright and Yoder 2003).

### 3.3. Assessment of Skeletal Pathology and the "Osteological Paradox"

The Osteological Paradox, introduced by Wood et al. (1992), elaborates on the indirect relationship between skeletal observations and inferred health in past populations. Age-at-death (selective mortality) and "individual frailty" play key roles in our understanding of paleodemographics (Wood et al. 1992). In traditional paleodemographics, individuals within a cohort (age group) who are most susceptible to morbidity/death make up the skeletal sample for that specific age range (Wood et al. 1992). This means bioarcheologists must make inferences about past population health based on the skeletons of individuals who may or may not be representative of their larger age group as a whole (Wood et al. 1992).

Wood et al. (1992) note that "disadvantaged" or "frail" groups within a cohort or larger population are more likely to die from an initial metabolic insult; whereas less "frail" individuals will have more signs of skeletal and dental pathology, indicating repeated recovery under maladaptive conditions. This is defined as the "Osteological Paradox", which states that a direct relationship between skeletal pathology and severity of illness cannot be directly observed. An individual who survives metabolic insult long enough to develop skeletal or dental indicators of stress is often better able to "buffer" stress than someone who succumbed to the same illness before signs of skeletal pathology could emerge (Wood et al. 1992). For this reason, age-at-death, individual resistance to stress, and "cultural buffering systems" (Goodman and Armelagos 1989), which may favor certain individuals over others, must be considered when examining stress in archaeological populations.

### 3.4. Dental Development and Enamel Formation

Odontogenesis refers to the process of tooth formation during fetal and early childhood development. Teeth begin as ectodermic germ cells in the developing embryo and form "buds" which develop into fully-formed teeth (Hillson 1996). Teeth have two primary sections: the crown, which is the visible portion of the tooth that lies above the gum line, and the root, which lies within the bony crypt of the maxilla or mandible. The primary (deciduous) dentition begins developing *in utero*; the first permanent molar is the only permanent tooth which begins forming while the fetus is still in the intrauterine environment (Hillson 1996). While this section focuses on the development and mineralization of the permanent dentition, it is important to note the relative timing of deciduous dentition when compared to that of the permanent teeth as the crowns of



*Figure 3.3.* Cross-section of human mandibular canine taken from Goodman and Rose, 1990 (modified from Rose et al. 1983, p. 283.) Reproduced with permission.

deciduous teeth form prenatally, while the adult dentition largely develops after birth when the child can be influenced by the external environment.

The primary dentition begins forming in utero between 14-16 weeks, with the development of the central incisors, followed closely by the deciduous first molars 15 weeks after fertilization (Hillson 1996). All teeth that begin forming in the intrauterine environment exhibit a "neonatal line" that can be observed histologically in cross-sections of the deciduous dentition and permanent first molar (Eli et al. 1989). This line demarcates pre-and-postnatal dentine and enamel formation, and results from the sudden, acute stress of live birth (Eli et al. 1989; Canturk et al. 2014). The thickness of this line can be correlated with the severity of the trauma at birth; in clinical settings, infants undergoing vaginal delivery have more prominent neonatal lines than infants delivered by Caesarian section (Canturk et al. 2014). The neonatal line is particularly useful in bioarchaeological and forensic cases, as it does not appear in stillborn infants (Canturk et al. 2014).

Dental tissue can be divided into three general categories: enamel, dentine, and cementum (Figure 3.3). Cementum coats the root of each tooth and helps anchor it to the periodontal ligament lining the tooth crypt (Hillson 1996). Enamel forms the hard, outer surface of the tooth crown. Mature enamel is composed of 96% inorganic material by weight, of which hydroxyapatite is the primary mineral (hydroxyapatite), and 4% organic materials, including water and proteins like amelogenin (Hillson 1996). Dentine, the inner layer of dental tissue, is composed of 70% inorganic hydroxyapatite and 20% organic proteins (collagen), and 10% water (Hillson 1996). The higher organic component of dentin makes it more malleable and more susceptible to diagenetic change

than enamel. Cementum is composed of an approximate 50:50 ratio of inorganic and organic material by weight (Hillson 1996). The dentin and enamel surround the pulp cavity, which contains blood vessels and nerves which help maintain the organic tissue of the tooth and provide sensory input to the dental arcade.

The permanent (adult) dentition forms during the first few years of life, with the exception of the first molar, which begins development in utero. Odontogenesis begins at the crown of the tooth and terminates at the root (Hillson 1996). Amelogenesis (enamel formation) refers to the secretion and deposition of the enamel matrix by specialized cells known as ameloblasts during tooth development (Hillson 1996; Hindle 1998). The deposition of enamel forms concentric bands (Striae of Retzius), visible on cross-sections of developed teeth (Figure 3). Perikymata appear as raised ridges on the crown surface and indicate the termination point of individual striae on the external enamel surface (Hillson, 1996). There is debate in dental anatomical literature about the exact definition of "perikymata", here, perikymata refers to the "raised ridge between two lines of imbrication" on the enamel surface (Roa and del Sol 2017). Several other important dental structures, including the cemento-enamel junction, are illustrated in Figure 3.3. Wilson bands are internally visible in cross-sections of the teeth, and often correspond with external hypoplastic defects on the enamel surface of the tooth (Goodman and Rose 1990).

The development of the mandibular central/lateral incisors and maxillary central incisors initiates around four to five months *post partem*; canines at approximately five months; lateral maxillary incisors around one year, with the third premolar, fourth premolar, and second molar developing sequentially during the second and third years of

life (Hillson 1996) (Table 3.1). Although age-at-completion varies for each tooth, development of the permanent dentition terminates in early adolescence with the exception of the third molar (Table 3.1).

Standard adult dentition includes 32 occluded teeth, with final root completion of

		Permanent Dentition			
	Calcification begins	Crown (enamel) completes	Roots complete	Eruption	
				Maxillary	Mandibular
Central incisors	3-4 mo	4-5 y	9-10 y	7-8 y	6-7 y
Lateral incisors	Maxilla: 10-12 mo	4-5 y	11 y	8-9 y	
	Mandible: 3-4 mo	4-5 y	10 y		7-8 y
Canines	4-5 mo	6-7 у	12-15 y	11-12 y	9-11 y
1st premolars	18-24 mo	5-6 y	12-13 y	10-11 y	10-12 y
2nd premolars	24-30 mo	6-7 y	12-14 y	10-12 y	11-13 y
1st molars	Birth	30-36 mo	9-10 y	5.5-7 y	5.5-7 y
2nd molars	30-36 mo	7-8 у	14-16 y	12-14 y	12-14 y
3rd molars	Maxilla: 7-9 y			17-30 y	
	Mandible: 8-10 y				17-30 v

*Table 3.1.* Table created by J. Lacerte based on data from the American Association of Pediatric Dentistry (adapted from Logan and Kronfeld 1933), showing enamel calcification and average crown completion times; mo= months, y= years.

the third molars occurring between the ages of 22-25 (Jung and Cho 2014). However, the third molars are developmentally variable; initial amelogenesis occurs anywhere between the ages of seven and thirteen and this tooth may be congenitally missing in the upper and/or lower dental arcade in individuals from both modern and bioarchaeological samples (Reid and Dean 2006; Jung and Cho 2014). Absence of the third molars is genetically variable and is therefore not considered a pathological condition.

Cuspal enamel formation should also be taken into consideration when examining teeth for LEH. Previous research has suggested that the formation period for cuspal enamel impacts the age estimates for enamel hypoplastic defects on the surface of teeth, as very early, cuspal perikymata can be "hidden" due to the concentric growth of tooth enamel (e.g.: Goodman and Song 1997; Reid and Dean 2000). Further research (Ritzman et al. 2008) confirmed that including cuspal formation times in the estimation of LEH age does alter the age-at-defect estimates observable on the surface of teeth.

Reid et al. (2008) reanalyzed cuspal enamel formation times for the Northern European and South African samples originally examined by Reid and Dean (2006). Cares-Henriquez and Oxenham (2019) make use of cuspal enamel formation times when developing their exponential regression equations for estimating age of enamel development. However, it should be noted that none of the formulae used to estimate age of tooth development have standards taken from modern or archaeological South American samples and are sourced from Northern European and Southern African samples (Reid and Dean 2006).

Dissertation research by Gaither (2008) attempted to develop dental eruption charts for Andean populations, but she does not include the precise estimates of crown formation times necessary to develop specific age-at-defect estimation formulae. While Gaither's (2008) dissertation provides an alternative to dental eruption charts based on European standards, there is still a lack of in-depth studies from the Andean region that use microscopic methods of dental analysis, or standards that look more specifically at crown (enamel) formation times in South American samples

# 3.5. Enamel Defect Etiology and Appearance

When amelogenesis is interrupted due to a stress event, defects form in the enamel surface. Several factors must be in place for an individual to reach the "threshold" (Figure 3.4) of ameloblast disruption for a particular tooth (Goodman and Rose 1990). As previously discussed, genetic "frailty" may play an initial role in the disruption of amelogenesis. However, poor nutritional intake combined with environmental stress plays a key role in pushing an individual past the "threshold line" of metabolic disruption (Goodman and Rose 1990).

Perikymata thickness varies across the crown surface (Hillson and Bond 1997). When examining enamel defects, the tooth is typically divided into thirds (Hubbard et al. 2009). The cervical third of the tooth is the portion closest to the cemento-enamel junction, and the last portion of the tooth to develop chronologically. The middle third of the tooth is often the portion of the enamel surface with the most defects (Goodman and Armelagos 1985; Hindle 1998). Finally, the occlusal or incisal third of the tooth has the "earliest" developmental perikymata, and the fewest developmental defects overall (Hindle 1998).



*Figure 3.4.* Diagram from Goodman and Rose (1990, p.75) illustrating the developmental threshold for the formation of enamel defects. Reproduced with permission.

A single perikyma takes between 6-12 days to form, with an average formation time of nine days (Reid and Dean 2006). Perikymata on the cervical third of the tooth are ~50 $\mu$ m in width, ~70 $\mu$ m for the middle third (mid-crown), and ~100 $\mu$ m for perikymata in the incisal third of the crown. It is important to note this progressive variation in width, as microscopic analysis of LEH has the potential to identify "abnormally spaced" perikymata which, if matched antimetrically or across tooth types, may represent stress events of a shorter duration than typical defects found on the enamel surface (Hassett 2012). This finding becomes significant when we consider total enamel defect width in comparison to individual spacing of perikymata (Hubbard et al. 2009), as defect width is not directly correlated with the number of perikymata contained within the defect due to variation in perikymata width across the enamel surface. A further explanation of the methods used to measure visible perikymata is available in the Materials and Methods chapter (Chapter 4).

Linear enamel hypoplastic defects are the most common form of hypoplastic enamel defects (Hillson 1996). These defects are associated with systemic metabolic disruption that occurs during crown formation (Hillson 1996; Marchewka et al. 2014). However, other types of enamel defects can occur, including non-linear pitting and horizontal grooves. While most LEH appear as "furrows" (Hillson 1996) on the tooth surface, some researchers have suggested that single, prominent perikymata of varying



*Figure 3.5.* Examples of short-term (right) and longer duration (right) linear enamel defects. The yellow lines/arrows indicate the approximate breath of the hypoplastic lesion.

thickness on the enamel surface can represent stress events of lesser duration (Hassett 2012). These single-perikyma (Figure 3.5) defects can be difficult to discern from "false"

hypoplastic defects (Goodman and Rose 1990) and should therefore be matched across tooth types or antimetric pairs to confirm etiology.

LEH are non-specific indicators of stress, as they cannot typically be associated with a specific pathological infection or stress-event (Guatelli-Steinberg and Lukacs 1999). Traditional studies of LEH on the enamel surface have examined these lesions and their relationship between major developmental milestones like infant weaning, which is often associated with periods of malnutrition and "weanling" diarrhea (Goodman and Rose 1990; Berbesque and Hoover 2018).

## 3.6. The "Weaning Hypothesis" and Developmental Sensitivity to Defect Formation

The history and severity of illness early in development impacts susceptibility to ameloblastic interruption. Infant weaning and its associated difficulties: diarrhea, febrile disease, and inadequate nutrition, is often discussed in the context of enamel defect formation (e.g. Goodman and Rose 1990). The curve in the "threshold line" (see Figure 3.4) represents the middle third of the tooth, which is the area with the lowest threshold for developmental enamel defects (Goodman and Rose 1990). It should be noted that the middle third of the crown generally develops on anterior teeth during the same period that is often typical of human infant weaning (between the ages of one and four, Ritzman et al. 2008). Therefore, the increased presence of enamel defects in this area does not necessary correlate directly with a particularly traumatic weaning period, rather, it may reflect the lowered metabolic sensitivity that occurs during this period of crown development due to increased external pressures on the developing infant (Hindle 1998). Alternatively, it may reflect a lowered threshold for lesion expression due to a variety of comorbid factors (Goodman and Rose 1990).

Additionally, different teeth have different metabolic "thresholds" for the formation of linear enamel defects. Dental studies (Goodman and Rose 1990; Guatelli-Steinberg et al. 2004; Tsukamoto 2003) have noted that mandibular canine teeth and maxillary incisors appear to be the most developmentally susceptible to enamel defects in both human and non-human primates. Many studies that examine LEH in archaeological populations employ antimetric pairs of teeth rather than single teeth to exclude the possibility of localized amelogenic interruption (Hindle 1998; Guatelli-Steinberg et al. 2004; Hassett 2012). Although rare, external trauma to the craniofacial area can cause "localized", unilateral hypoplastic lesions (Hindle 1998), but these can be easily identified from the associated trauma. Different teeth from the same individual with overlapping development can also be used (Hassett 2012). Finally, much of the primary literature on enamel formation times come from modern, clinical contexts and do not always account for temporal or regional variation in tooth formation (Hassett 2012). More recent studies (Reid and Dean 2006) have shown developmental differences between modern populations, given that this variability could also be assumed for human populations in the past, this possibility warrants further investigation (Martin et al. 2008).

Bioarchaeological research employs several different methods of determining the age at which enamel defects occur. Martin et al. (2008) tested the effectiveness of the method developed by Reid and Dean (2000) and earlier regression equations developed by Goodman and Rose (1990). The results of this analysis indicate there is a significant difference between the Reid and Dean (2000) decile charts and regression equations (Martin et al. 2008). However, the authors note that a difference of one-to-four months in terms of childhood development is unlikely to make a large impact on the overall

reconstruction of childhood growth and development (Martin et al. 2008). Additionally, as stress-events like weaning are culturally (and individually) variable, and take place over an extended period of time, it is unlikely that slight variations in the first appearance of LEH are significant at the population level (Martin et al. 2008).

While the Reid and Dean (2006) method is often preferred over the Goodman and Rose (1990) regression formulae in recent literature (e.g. Ritzman et al. 2004; Martin et al. 2008), Martin et al. (2008) note that if available, population-specific decile charts are the best method, as they account for cross-cultural variation in enamel formation times. More recently, exponential regression equations have been published in an attempt to address some of the errors inherent in both the linear formulae methods and the decile chart method (Cares-Henriquez and Oxenham 2019). The following chapter, Materials and Methods (Chapter 4), will outline the specific methods used to measure LEH from images captured using the Scanning Electron Microscope.

### CHAPTER FOUR: MATERIALS AND METHODS

### 4.1. Skeletal Data Collection

Skeletal and dental data were collected at the *Museo Nacional de Arqueología*, *Antropología e Historia del Perú* in Pueblo Libre District, Lima, Peru. Prior to casting, a basic biological profile was created for each individual in the sample. Photographs of the mandibular/maxillary dental arcade, skull, and notable pathology were also taken prior to casting (see Appendix C for photographs). All dental measurements and observations were recorded by J. Lacerte. Dr. S. Gauld (Professor Emeritus, Santa Monica College) assisted with some long bone measurements and assessment of pathology. The biological profile included basic sex and age-at-death estimation using morphological characteristics of the skeleton (following Buikstra and Ubelaker 1994). Individuals were selected on the basis of reported skeletal completeness from museum records, and a visual survey of the remains housed at the *Museo Nacional de Arqueología* conducted prior to the start of data collection by Dr. A. Nelson and Dr. S. Gauld.

Sex	n	Mean Age	SD	SE	#Teeth
Female	10	33	8.317	2.630	94
Male	11	31	6.856	2.067	116
Adolescents	5	14	0.758	0.339	70
Total	26	29	9.746	1.911	280

*Table 4.1.* Table illustrating the extent of original data collected for this thesis, n= number of individuals, SD= standard deviation, SE= standard error of the mean, #Teeth= total number of teeth cast per sex category, overall totals for the sample are included in the bottom row.

In some instances, metric sex estimation of the skeleton was performed using prior population-specific sex estimation formulae developed by the author (Lacerte and Slank 2018) from a population from Southern coastal Peru (Raxter et al. 2006). This method was used as a secondary means of sex estimation to check the results of visual analysis. Adolescent age-at-death was estimated by examining the progression of dental root development (as assessed when loose teeth were available) and epiphyseal union following White and Folkens (2005). Ectocranial suture closure (Buikstra and Ubelaker 1994; White and Folkens 2005) was used as an additional age estimation method in adults when the presence of soft tissue, hair, artificial cranial modification, and taphonomic damage did not affect observation of cranial sutures. Finally, maximum femoral length and femoral head diameter were recorded for adult individuals and adolescents with fused/fusing proximal and distal epiphyses.

## 4.2. Measuring and Recording Dental Indicators of Stress

Bioarchaeological research on enamel defects typically involves two primary methods of recording defects: visual and microscopic analysis. Visual analysis often includes the use of a hand-held magnifying device to help observe the surface of the tooth. The distance from the cemento-enamel junction to the occlusal wall of a defect is measured using calipers (following Buikstra and Ubelaker 1994). This gross macroscopic method is useful for field analysis, but individual perikymata (which are used to determine defect duration) cannot be observed in-situ. Hence, dental impressions of the buccal/labial enamel surface were taken from these teeth to be cast and imaged after returning to the University of Western Ontario from Peru.

Linear hypoplastic defects on the enamel surface of the teeth were recorded visually with the aid of a 5x hand-held magnifying glass following Buikstra and Ubelaker (1994). LEH position on the tooth was recorded from the cemento-enamel junction to the cervical wall of the defect (following Guatelli-Steinberg et al. 2004). Dental morphology, including mesiodistal/ buccolingual diameter and crown height were also recorded. All dental measurements (mm) were taken with Mitutoyo® (500-196-30) digital calipers,

with a precision of two decimal points. Other types of nonlinear hypoplastic defects (pitting, missing enamel etc.) were recorded following Buikstra and Ubelaker (1994).

Selection criteria for taking dental impressions included at least two intact antimetric pairs of adult teeth (i.e.: incisors, canines) from either the maxillary or mandibular dental arcade (Guatelli-Steinberg et al. 2004). Following Temple et al. (2013), heavily worn teeth were excluded from casting if they contained <70% of estimated crown height. Additionally, teeth with dental calculus covering over 50% of the buccal crown surface were excluded. Adolescent individuals were included in the sample if all permanent teeth except the third molars were present and in occlusion. To avoid possibly biasing sample collection by selecting younger individuals with less dental attrition, individuals were selected based on reported skeletal completeness.

The relative completeness of these skeletons allowed for additional visual and osteometric data to be collected by Dr. S. Gauld. Visual analysis of LEH on the teeth was not completed until other osteometric data was collected. The data sheets used to collect sex, age, and dental information were modified by J. Lacerte and Dr. A. Nelson from Buikstra and Ubelaker (1994). Prior to measuring LEH on the surface of the teeth, morphological dimensions of the teeth (crown height, buccolingual, and mesiodistal diameter) were taken, along with visual observation of dental wear and carious lesions.

Overall, the sample includes 10 females, 11 males, and 5 adolescent individuals of indeterminate sex (see Appendix A) for a total of 26 individuals (Table 4.1). While a sample size of 26 meant more attention and care could be taken during skeletal assessment, data collection, and the creation of dental impressions, the smaller sample size of this thesis raises important methodological and interpretive questions, particularly for the statistical analysis of the data presented. A discussion of the limitations of sample size for this thesis is presented in Chapter 6.

Originally, 280 dental impressions and casts were collected/created which included incisors, canines, and premolars from both the maxillary and mandibular dental arcades (see Appendix A). However, due to the magnitude of casts and time constraints of SEM imaging for this thesis, a number of 60 (30 antimetric pairs) from the original 280 dental casts were selected for SEM imaging. Central maxillary incisors and mandibular/maxillary canines were selected for analysis as these teeth are often the most developmentally sensitive (e.g. Hillson 1996; Guatelli-Steinberg et al. 2004). While at least one antimetric pair of teeth from each of the 26 individuals examined were analysed for this thesis project, the author of this thesis hopes to expand the analysis in the future, hence the large number of dental casts (n= 280) originally created.

The sample ranges between 12-50 years of age, with a mean age of 29. These age estimates were determined on the basis of common morphological methods of age estimation (Buikstra and Ubelaker 1994; White and Folkens 2005) for both adults and adolescents. In addition, it is recognized that these figures represent the distribution of ages of this study sample, and not the age distribution for the larger site of Rinconada Alta.

## 4.3. Temporal Distribution of Rinconada Alta Sample

The cemetery sectors at Rinconada Alta experienced repeated use throughout Andean prehistory, with modern and Inca period material often overlaying previous cemeteries (Diaz 2011). Prior dissertations (Salter-Pedersen 2011; Marsteller 2015) assign conflicting time periods from the Late Intermediate period (LIP) (Marsteller 2015)

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and Inca period (Salter-Pedersen 2011) to their samples from Rinconada Alta. Therefore, a detailed overview of approximate dates for the sample needs to be discussed to avoid potential confusion in this present thesis. Of the 26 individuals examined for this thesis, there are individuals from 3 of the 6 cemetery sectors (Sectors I, II, VI) at the site (see Table 4.2). All individuals selected for this thesis sample were categorized as *material óseo* or skeletonized remains in the Rinconada Alta Site Inventory (based on the excel catalogue of the *Museo Nacional* and customized and updated by A. Nelson 2017).

Sector	I	II	IIA	IIAE	IV
N	2	4	4	15	1
Period	Inka	LIP-Inka	Inka	Inka	LIP-Inka

*Table 4.2.* Distribution of sample (n=26) for the current thesis within the larger cemetery sectors and subsectors at Rinconada Alta. LIP= Late Intermediate period

Sector I is the "principal nucleus" (Ruiz and Guerrero 1996) of the cemetery, and consists of Inca period burials organized by family/ethnic group/social status; there are two individuals from this sector in the current sample (Table 4.2). Sectors IIA and IIAE are extensions of cemetery Sector II (Diaz 2011; Salter-Pedersen 2011). Sector II of the cemetery has two layers of burials: one that clearly dates to the Inca period, and a preceding layer that represents the end of the LIP and beginning of the Inca period (Ruiz and Guerrero 1996). The burials from Subsector IIA, from which IIAE is an extension (Salter-Pedersen 2011), contain Inca period burials in a variety of positions and orientations and include Yschma, Inca, and Yaoyos ceramic styles, suggesting a variety of burial practices/ethnic identities were present during the Late Horizon (Ruiz and Guerrero 1996).

The majority of the sample for this project is taken from Sector IIA, sub-sector IIAE. Sector IIA largely consists of individual burials with some infant, child, and adult

*fardos* or "mummy bundles" (Diaz 2011) although no *fardos* were used for the current sample. There is some evidence at the site of "retainer burials", a practice in which the remains of elite individuals are accompanied by burials of lower social rank of similar age or sex (Diaz 2011). Diaz (2011) suggests, based on the presence of these burials, that there was some social differentiation within the Yschma culture prior to Inca conquest. The one individual (RA 905) from Sector IV in the current sample is from layer C, which is dated to the end of the LIP and beginning of the Late Horizon (Inca period). Sector IV also contains osteological and archaeological material from the Early Intermediate period (AD 100-600) Lima culture (Díaz and Guerrero 1997, 1998). While there is some overlap in the sample between the LIP and Inca period, it is uncertain whether the five burials (see Figure 4.2) from these admixed sectors date to the LIP or Inca periods respectively. The remaining twenty-one individuals selected for this study are from sectors/sub-sectors that likely date to after Inca conquest, (AD 1470) although admixture is present throughout Sector II (Diaz 2011; 2015).

Given the biological distance analysis provided by Salter-Pedersen (2011) and isotopic data presented by Marsteller (2015), it is unlikely that the occupants of Rinconada Alta were resettled after the Inca conquest. Therefore, while there may be some admixture of burials from different time periods represented in this sample, it is likely that the majority of burials are from a post-Inca conquest context and will be considered as such for the purposes of this thesis research.

## 4.4. Dental Impressions, Casting, and SEM Preparation

Dental impressions were taken using 3M Light Body Polyvinyl siloxane. TransPort<sup>TM</sup> Handheld Disposable Impression Syringes (Figure 4.1) were used to apply the polyvinyl siloxane directly to the enamel surface. Prior to this step, some teeth were gently cleaned with a cotton swab and regular tap water to remove loose surface debris. This method of taking impressions of the dentition was developed during previous research on human remains from the Southern coastal site of Chiribaya Baja (Barrett and Mast 2016).

Polyvinyl siloxane works best at temperatures between 15-25 °C with <50% humidity and has an approximate setting time of 5 minutes once applied to the tooth



*Figure 4.1.* Diagram illustrating the steps taken during data collection by the author of this thesis, which include: taking dental impressions, casting/mounting technique, and SEM imaging. Photo credits: Joanna Motely, 2018 and Jessica Lacerte, 2019.

surface (Fiorenza et al. 2009). Humidity in excess of 50% increases drying time

(Fiorenza et al. 2009). The dental impressions were taken in a covered tent outdoors;

average humidity in Lima for the month of July is 75%, with temperatures between 15-

19°C (worldweatheronline.com ). Therefore, the dental impression material was removed from the tooth surface 8 to 10 minutes after initial application to give the impressions sufficient time to dry.

Positive casts of the teeth were created at the Zooarchaeology laboratory in the Department of Anthropology at the University of Western Ontario. Struers Epofix<sup>TM</sup> cold-mounting resin was used to create the casts. The EpoFix<sup>TM</sup> resin consists of a resin epoxy and a liquid hardener, which are mixed in a ratio of 25:3 volume by weight. To improve visibility under a light microscope for the next stage of analysis, several drops of Liquitex<sup>®</sup> Professional Acrylic Ink (#317 Phthalocyanine green) were added to the clear resin mixture. This was stirred into the resin admixture with a wooden spatula until the color was evenly distributed. To remove air bubbles from the resin, the dyed resin mixture was placed in a vacuum impregnator for 10 minutes. The dental impressions were placed into setting trays made from CraftSmart<sup>®</sup> Plastalina Modeling Clay to prevent the liquid resin mixture from overflowing in the dental impressions and left to cure for approximately 24 hours. After 24 hours, the hardened casts were separated from the polyvinyl siloxane impressions and individually re-bagged.

The dental casts were shaped using a Dremel tool to remove excess casting material and sharp edges so the anterior surface of the casts would lay flat when mounted. Prior to mounting, the casts were sonicated using a New Trent CD3800 Ultrasonic Cleaner for 180 seconds in 70% alcohol solution to remove surface debris and were then allowed to dry for 5-10 mins. Thorough cleaning of the samples before imaging is essential; small particulates, sebum, and dust can adhere to the SEM aperture and create aberrations in the final lens image (Insepov et al, 2008).

Antimetric pairs of casts were then mounted on Ted Pella Aluminum SEM pin stubs using graphite tape and colloidal graphite solution. Additionally, a line of silver paint was added to "ground" the non-conductive casts to the aluminum stub, which prevents charge buildup. An arrow was scored into the middle of the aluminum stub using a scalpel to indicate the location of the CEJ relative to the dental casts and to make the orientation of the tooth easier to identify once inside the SEM chamber.

Teeth cast from the left side of the dental arcade were mounted on the left side of the arrow, and teeth cast were mounted on the right. All teeth were mounted with the cervical third of the tooth facing upwards, regardless of position in the mandibular or maxillary arch. This was done to keep the SEM images consistent between all dental casts, and to avoid confusion when labelling images. The samples were then placed in a SPI-MODULE<sup>TM</sup> Sputter Coater and coated with gold-palladium alloy to make the samples electron dense and reduce charging. Gold produces many secondary electrons when interacting with the electron beam and is widely used as a material to sputter-coat non-conductive samples (Insepov et al, 2008).

## 4.5. SEM Imaging

After sample preparation, the stubs were inserted into the AMRAY 1820-I scanning electron microscope in the SEM lab at Eastern Michigan University. While there are several different ways to generate an electron beam, the AMRAY 1820-I uses a Lanthanum Hexaboride (LaB<sub>6</sub>) crystal held in place by tungsten "hairpins", which is heated to produce an electron cloud. The voltage on the thermal emitter generally ranges
from 0.1-40 Kilovolts (Dunlap and Adaskaveg, 1997). All teeth were imaged between 5-8 keV to increase the overall resolution of the samples.

The electron cloud generated by the thermal emitter is focused through a series of electromagnetic condenser lenses (Dunlap and Adaskaveg 1997). Other lenses in the SEM correct for electron beam misalignment and astigmatism. Astigmatism, indicated by a "stretching" of the final lens image, is caused by an uneven distribution of iron within one of the condenser lenses (Dunlap and Adaskaveg 1997). This can be corrected by adjusting the "Stigmater" (a series of eight smaller magnets) so that the beam resumes its circular shape.

When examining the casts for hypoplastic defects, the user needs an image with good topographic contrast. In the SEM, this relates to how the electron beam interacts with samples with highly irregular surfaces. Primary electrons from the beam hit the sample and generate secondary electrons by "knocking off" electrons within the sample. These secondary electrons are used to generate the final lens image. Backscattered electrons "bounce off" the sample and provide additional image resolution needed for specimens with irregular surfaces (Dunlap and Adaskaveg 1997). The *collector bias* on the SEM's Eherhart-Thornely detector can be lowered so that more backscattered electrons are processed through the photomultiplier. This results in an image with better topographical contrast (Dunlap and Adaskaveg 1997). In this current study and in previous research (Barrett and Mast 2016; Lacerte et al. 2016), lowering the collector bias increased surface visibility of enamel defects when imaging resins casts using the AMRAY 1820-I.

Sample images were taken at several different magnifications. Overview images of the entire crown of the tooth were taken at  $\sim 10x$ , with higher magnification images between 20-50x. At lower magnifications a 1mm scale was included in each image for higher magnification a scale of 100 microns (µm) was used.

## 4.6. ImageJ Analysis

The SEM images were measured using ImageJ (v.2.0.0), an open-source image processing software (https://imagej.nih.gov). Overview images were measured using the "Label and Measure" plugin from the midline of the cemento-enamel junction to the occlusal wall of the observed defect (Figure 4.2), closely following the visual method of recording LEH recommended by Buikstra and Ubelaker (1995). The measurements were added to an ExcelMac 2016 file for further analysis.

Following (Guatelli-Steinberg 2004; Hassett 2014; Barrett and Mast 2016), closeup images of surface perikymata were measured sequentially from the occlusal wall to



*Figure 4.2.* SEM overview image of RA 720 left maxillary canine ( $\sim$ 10x), numbered lines represent the number of measurements from the cemento-enamel junction to occlusal side of suspected hypoplastic lesion. per category. The yellow lines from the CEJ to occlusal wall of the defect represent the number of observable defects that were matched across tooth types, the reader may note another faint line, which the author was unable to match across the other side of the tooth.

the cervical wall of a defect. Hassett (2014) suggests the use of a "moving average" based on surrounding perikymata to estimate perikymata width for that section of the tooth. Hassett (2014) notes that perikyma widths more than two standard deviations from the "moving average" likely represent interruptions to enamel formation. Following Barrett and Mast (2016), all perikymata surrounding a suspected defect were measured. When the occlusal wall of a defect could not be easily defined when examining smaller defects without a deep "furrow" in the enamel surface, the number of abnormal perikymata alone was used to determine defect duration.

While defect width remains a relatively good indicator of defect duration (Hillson and Bond 1997; Guatelli-Steinberg 2004), it is less effective than measuring total perikymata counts within a defect, due to variation within perikymata width on different thirds of the tooth. For this reason, images of defects with heavily worn or no observable



*Figure 4.3* SEM image of enamel surface of RA 888 maxillary right canine (100  $\mu$ m), perikymata surrounding defect have been obliterated, but perikymata within the defect remain visible. The yellow arrows indicate the "trough" or imbrication line prior to each "peak" or perikyma on the enamel surface within the defect.

perikymata within an observable defect were excluded from this portion of image analysis. Images with worn perikymata were still included in Image J analysis if they contained observable perikymata within the defect itself (Figure 4.3).

The duration of metabolic disruption was then estimated from the Image J measurements of perikymata using an average of 9 days (following Reid and Dean 2006; Barrett and Mast 2016) for perikymata formation. These results were then compared to those for the site of Chiribaya Baja (Barrett and Mast 2016).

### 4.7. Enamel Defect Age Estimation

The visual and microscopic LEH defect measurements were converted into age estimates using formulae developed by Goodman and Rose (1990). Since the publication of these formulae, several other methods of estimating age-at-defect occurrence have been suggested (Reid and Dean 2006; Martin et al. 2008).

The Reid and Dean (2006) method uses "decile charts" developed from European and South African samples to estimate age based on location within a "decile" of the adult tooth. These teeth were sectioned, so exact enamel formation times could be estimated from the internal structure of the enamel (Reid and Dean 2006). The teeth examined in the Reid and Dean (2006) study were in relatively good condition; when dental wear was present, the percent of missing enamel was estimated using complete teeth of that tooth type from the rest of the sample.

Martin et al. (2008) found a statistically significant difference in age estimation using formulae from Goodman and Rose (1990) vs. the decile chart method suggested in Reid and Dean (2006). However, given that hypoplastic defects often appear concurrently with infant weaning, which occurs gradually, a difference of "1 to 4 months" which, in the opinion of Martin et al. (2008), is often likely to be "biologically insignificant" in most instances of metabolic disruption (p.364). For the purposes of this thesis, the Goodman and Rose (1990) formulae were selected due to the presence of wear on almost all of the teeth examined, due to the fact that even young individuals in the sample exhibited a mild to moderate amount of dental wear and carious lesions. More recently developed exponential regression equations published by Cares-Henriquez and Oxenham (2019) were also used to estimate defect age, as these formulae take into account both dental wear and cuspal enamel formation times, something which the linear Goodman and Rose (1990) formulae have been critiqued for not taking into account in the past (Goodman and Song 1999; Ritzman et al. 2008; Martin et al. 2008).

The severity of dental wear between antimetric pairs of teeth from Rinconada Alta was sometimes asymmetrical. Given the lack of population-specific information on enamel formation from archaeological populations in the pre-Columbian Andean region, the Goodman and Rose (1990) formulae provide good estimates of age-at-metabolic interruption without the need to calculate the specific amount of enamel lost for each pair of teeth examined.

For purposes of methodological comparison, the defect age estimates produced by the Goodman and Rose (1990) and Cares-Henriquez and Oxenham (2019) formulae were both used to calculate the frequency of defect occurrence between birth and the age of seven in the Rinconada Alta sample. As discussed in Chapter 3, this age range reflects the upper and lower limits of enamel formation time across all anterior teeth (incisors, canines) selected for this study. To determine the age at which enamel defects most frequently occurred in the sample, stress episodes (LEH) from antimetric pairs were matched across tooth types (if multiple tooth types from the same individual were present) and counted for all individuals in the sample (n= 26). The average number of defects per individual was also determined; first they were counted individually to compare the total, unpaired number of LEH per individual to the Puruchuco-Huaquerones sample (Murphy 2004) and then paired to reveal the true number of stress episodes per individual.

### 4.8. Dental Health and Stature

The frequency of oral pathology in the current sample was compared to published results from Puruchuco-Huaquerones (Murphy 2004). This included the average number of carious lesions, antemortem (prior to death) tooth loss, and dental abscesses between adult males and females in the Rinconada Alta and Puruchuco-Huaquerones samples. Differences in oral health between populations can be associated with diet, social status, and sex-based differences in food consumption (e.g. Lanfranco and Eggers 2012). Therefore, a comparison of oral health is a good measure of any potential differences in overall health and diet between the coastal Peruvian sites examined for this thesis.

Although Salter-Pedersen (2011) previously recorded dental pathology and adult stature at Rinconada Alta, this thesis revisited these two measures of health. No population-specific regression equations for estimating adult stature were available at the time of Salter-Pedersen's (2011) dissertation publication. Salter-Pedersen instead used the revised Mesoamerican stature formulae presented in Del Angel and Cisneros (2004) which were calculated from tables presented in Genovés (1967). The current study used the Del Angel and Cisneros (2004) formulae as well as stature formulae derived from the Chiribaya Baja sample (Lacerte and Slank 2018) in an attempt to see if the use of more population-specific formulae altered the average stature results for the Rinconada Alta sample when compared to Salter-Pedersen's (2011) earlier estimates. The Chiribaya formulae were originally derived using the Revised Fully method (Raxter et al. 2006) of stature estimation from complete skeletons from Chiribaya Baja (Lacerte and Slank 2018). Maximum femoral length was measured using a field osteometric board for adult males/females with intact femora in the current Rinconada Alta sample. A total of 9 males and 9 females were included in this portion of the analysis. Average stature from the current Rinconada Alta sample was also compared to published average stature for Puruchuco-Huaquerones, which used the unrevised Mesoamerican formulae presented in Genovés (1967) (Murphy 2004).

## 4.9. Statistical Data Analysis

To test whether average numbers of LEH per male (n=11), female (n=9), and adolescent (n=5) sub-groups were statistically significant, a non-parametric test, the Kruskal-Wallis one-way ANOVA on ranks was performed. Although a D'Agostino-Pearson  $K^2$  omnibus test for normality (DAPT) was initially run on this dataset to test for normal distribution of the sample, a non-parametric test was eventually chosen to analyze average number of LEH between sample sub-groups due to the smaller numbers of individuals examined for this thesis.

A paired t-test was performed in SPSS (v. 20) to determine whether any statistically significant differences existed for age-at-defect occurrence estimation employed in this current research (Goodman and Rose 1990; Cares-Henriquez and

Oxenham 2019). A paired t-test was selected, as the age estimates produced by both formulae used the same set of Image J measurements. Prior to this, the data was tested for normality using a Shapiro-Wilks test.

In order to test whether the CEJ/defect measurements from the Image J software were replicable, intra-observer error rate was calculated using paired t-tests in SPSS (v. 20) by repeating these measurements on a number of randomly-selected overview images. The results for the frequency of defect occurrence, including average number of defects per individual, are presented in the following chapter (Chapter 5). Additional results, including comparison of average number of defects between males and females and adults/adolescents are also presented, along with intra-observer error for the sample. Finally, the average duration of metabolic disruption in the Rinconada Alta sample is presented, along with an analysis of defect duration and comparison to the Chiribaya Baja sample evaluated by Barrett and Mast (2016).

### **CHAPTER 5: RESULTS**

#### 5.1. Introduction

This chapter focuses on the results of the analysis of LEH at the site of Rinconada Alta. Previous research at Rinconada Alta (Salter-Pedersen 2011) suggested that the population at Rinconada Alta experienced more stress than that at the nearby site of Puruchuco-Huaquerones (Murphy 2004; Williams 2005). This chapter will also test this assumption in light of more refined LEH timing and duration analysis, the implications of which will be discussed in the following Discussion (Chapter 6). Finally, two methods of age estimation for LEH will be compared to test whether there are significant differences in age estimation between the two methods. Additionally, this chapter examines potential differences in stress by comparing the duration of enamel growth disruption between the site of Rinconada Alta to the Late Intermediate period (AD 900-1300) site of Chiribaya Baja on the Southern coast of Peru. The data for the enamel defect age estimation portion of the analysis is listed in Appendix B.

#### 5.2. Analysis of Intraobserver Error

Intra-observer error was tested on a randomly selected sample of SEM images of the dental casts in ImageJ. Twenty-six measurements of distance from the CEJ to the defect were repeated on five pairs of dental casts from the current sample following the original method of Image J measurement described in Chapter 4. A paired t-test in SPSS (v.20) was used to calculate whether there was a significant difference between the two sets of observations taken by the author. At a significance level of (p>0.05) the results found that there were no statistically significant differences (p=0.854) between the two sets of measurements. This finding is similar to those reported by Hassett (2012; 2014) who reported no statistically significant intra or inter-observer error of enamel defect measurements using microscopic methods of analysis.

### 5.3. Differences in Visual vs. SEM methods

The total number of individual *unpaired* LEH observations for all 60 teeth examined in this study using visual methods of analysis (following Buikstra and Ubelaker 1994) was 84. The total number of individual *unpaired* LEH observations using the Scanning Electron Microscope was 145. The increased scale of observation using the SEM increased the number of smaller, "short term" enamel defects that were not observable with the naked eye, therefore increasing the total number of observations.

In some instances, SEM analysis can also help correct erroneous observations made in the field. In one instance, one set of teeth (RA 288) was recorded as having LEH in the field. However, under higher magnification, the defects were revealed to be surface debris and enamel scoring from wear. While the benefits of microscopic means of



*Figure 5.1.* Pair of SEM images showing a labial view of the left and right antimetric pair of Maxillary Central Incisors from RA 288. Note the loss of features on the enamel surface, casting aberrations, and cracks/surface scoring visible in the images.

analysis are well-established (Guatelli-Steinberg et al. 2004; Hassett 2012; 2014) the difference in number of LEH observations between these two methods has important methodological implications when attempting to compare LEH counts between sites/projects.

## 5.4. Analysis of LEH by Age and Sex

Using the SEM images, the number of defects per individual and subgroup (female, male, adolescent) was calculated by matching defects across antimetric pairs and if present, tooth types if more than one pair of dental casts were examined per individual. Defects were considered to be the from the same stress event if they fell within ~0.5 years of each other. Of the 26 individuals examined, there were 11 males, 10 females, and 5 adolescents with erupted permanent dentition. Of the 26 individuals examined, 1 adult female (RA 288), had heavily worn labial enamel and no microscopically observable LEH. This individual was excluded from additional from the subsequent datasets and analysis as the wear made it impossible to be sure if the lack of LEH or surface perikymata was real or not. Therefore, a total of 25/26 (96.2%) individuals from the entire original sample had observable LEH. From a total of 60 dental casts (30 antimetric pairs), a total of separate 58 stress events were observed for all individuals.

# Stress Episodes	Males	Females	Adolescents	Total
0	0	1	0	1
1	3	3	2	8
2	5	1	1	7
3	1	3	1	5
4	2	2	0	4
5	0	0	1	1

*Table 5.1.* Chart illustrating the range of stress events per individual by sub-group for this thesis.

The number of stress events (paired LEH) per individual (n= 26) ranged from 0-5 (see Table 5.1). Of note, only one subadult (RA 1041A) had 5 separate observable LEH (stress) events (Table 5.1).

	Ν	Stress Episodes	Mean	SD
Male	11	24	2.2	1.08
Female	9	22	2.4	1.23
Adolescents	5	12	2.4	1.67
Total	25	58	2.3	1.22

*Table 5.2.* Chart illustrating the total number of stress events for this thesis, including average number of stress events for males, females, and adolescents.

After the total number of stress events for the sample was determined, the average number of paired defects per individual (i.e.: number of childhood stress events) was calculated using ExcelMac 2016. The average number of LEH per individual for the entire sample was 2.3 (Table 5.2). To test whether there was a significant difference in the average number of stress events between adult males, females, and adolescents, a D'Agostino-Pearson K2 omnibus test (DAPT) for normality was performed using R Studio (v. 3.1.2) to help determine whether a parametric or non-parametric test was suitable for the analysis of the dataset.

As the dataset contained many repeated values, the DAPT normality test was favored over other the Shapiro-Wilk normality test (Royston 1995). This test was selected for its sensitivity towards both skewness and kurtosis in smaller datasets (Yam and Sim 2010). Unlike other more common tests for normality, The DAPT test is not readily available in common statistical software programs like SPSS (Yap and Sim 2010). For this reason, the open-source R statistical package was used to run the test for normality. The formulae used to run the DAPT test was taken from Zar (1999) and modified by C. Barrett in 2012. The results of the DAPT test confirmed the null hypothesis that the sample (LEH counts) was normally distributed (p=0.4275) with a confidence interval of 95% (p>0.05). However, as the sample size is relatively small (n=25), and the number of sub-groups (adult males, females, and adolescents) divides the dataset into even smaller groups, a non-parametric Kruskal-Wallis one-way ANOVA (see Table 5.2) of ranks was performed

					Test Stati	stics <sup>a,b</sup>	
	Sex	N	Mean Rank				
IEH #	1	٩	13.83	1			
	-	, ,	15.05		Chi-Square	.203	
1	2	11	12.41		df	2	
	3	5	12.80		Asymp. Sig.	.903	
	Total	25			a. Kruskal Wallis		
	Total	25		1	Test		
					b. Groupin Variable	g : Sex	

*Figure 5.2.* Output showing the results for the Kruskal-Wallis test in SPSS (v. 20). 1= female, 2= male, 3= adolescents. N= number of individuals included per sample group.

in in SPSS (v. 20) to test whether there were any significant differences between average number of LEH for the three sample groups (males, females, and adolescents). Figure 5.2 illustrates that the p-value= 0.903 at a confidence interval of 95% (p>0.05); suggesting that there are no statistically significant differences in the number of stress events experienced by males, females, and adolescents in the sample.

### 5.5. Timing of Enamel Growth Disruption

For this portion of the analysis, defect measurements taken from overview images of the antimetric pairs of teeth were converted into defect age estimates using the Goodman and Rose (1990) and Cares-Henriquez and Oxenham (2018) formulae (Figure 5.2). A total of 58 teeth (29 antimetric pairs) from 25 individuals were examined. Once event age estimates were obtained, the number of individuals with at least one LEH per age category was calculated (see Appendix B). The age categories ranged from birth to seven years and were divided into half-year increments (Table 5.3). The age of seven represents the completion of the crown of the canine teeth (Anthonappa and King 2015). An individual was included if they had *at least one* LEH that occurred within a given age range. Therefore, the percentages listed in Table 5.3 represent the number of individuals



Timing of LEH episodes between methods

*Figure 5.2.* Graph illustrating the number of individuals with LEH per half-year intervals using the Goodman and Rose (1990) and Cares-Henriquez and Oxenham (2019) formulae.

		Goodman an	d Rose (1990)		Cares-Henriquez and Oxenham (2019)				
	Le	eft	Ri	Right		eft	Ri	Right	
Age	N1	L%	N2	R%	N3	%	N4	R%	
0.0-0.50	0	0.0	0	0.0	0	0.0	0	0.0	
0.51-1.00	0	0.0	0	0.0	0	0.0	0	0.0	
1.01-1.50	1	4.0	0	0.0	0	0.0	0	0.0	
1.51-2.00	1	4.0	2	8.0	2	8.0	2	8.0	
2.01-2.50	4	16.0	6	24.0	3	12.0	0	0.0	
2.51-3.00	9	36.0	8	32.0	9	36.0	11	44.0	
3.01-3.50	11	44.0	11	44.0	11	44.0	11	44.0	
3.51-4.00	14	56.0	14	56.0	13	52.0	13	52.0	
4.01-4.50	6	24.0	8	32.0	10	40.0	11	44.0	
4.51-5.00	7	28.0	6	24.0	6	24.0	7	28.0	
5.01-5.50	6	24.0	7	28.0	1	4.0	0	0.0	
5.51-6.00	1	4.0	0	0.0	0	0.0	0	0.0	
6.01-6.50	0	0.0	0	0.0	0	0.0	0	0.0	
6.51-7.00	0	0.0	0	0.0	0	0.0	0	0.0	

*Table 5.3.* Table showing number of individuals with LEH per age category (n/25) for the left and right sides of the dentition using the Goodman and Rose (1990) and Cares-Henriquez and Oxenham (2019) formulae. N1-4= number of individuals with at least one defect per age category.

with *at least one* stress episode per age category divided by the total number of individuals in the sample (n=25).

Differential wear on some of the antimetric pairs meant that in a few cases, some stress episodes could not be observed on both teeth of the same type or across multiple antimetric pairs from the same individual. Additionally, age scores for the right and left side were not always exactly the same, meaning there was some overlap between the discrete age categories in Table 5.3. For example, an individual might have a defect on the left canine that occurred at the age of 4.4, while the same defect on the right canine might be aged as 4.5. For this reason, the right and left sides of the dentition are listed separately in Table 5.3. Issues with precision related to the determination of defect age categories will be discussed in Chapter 6. Using both the Goodman and Rose (1990) and Cares-Henriquez and Oxenham (2019) formulae, the highest percentage of LEH episodes occur between 3.51 and 4.0 years of age, across both formulae and sides of the teeth (Table 5.3). The slight difference between the height of the "peaks" seen on the graph (Figure 5.2) can be attributed to differential wear on the left and right sides of the teeth, potentially obscuring some unilateral LEH observations.

A paired, two-tailed t-test was performed in SPSS to determine whether there was a significant difference between the two methods of aging enamel defects. Prior to performing this analysis, the data was testing for normality using a Shapiro-Wilks test in SPSS and found to be normally distributed. The results of the t-test suggest (p>0.05) that there is a statistically significant difference (p=0.007) between the two methods of defect age estimation used in this analysis. The average ages produced by the Goodman and Rose (1990) formulae (averaged for the right and left sides of the dental arcade) are approximately 40.2 days (1.3 months) older than those estimated by Cares-Henriquez and Oxenham (2019). A discussion of the limitations of both methods and the results of this comparison are presented in the following chapter (Chapter 6).

## 5.6. Duration of Metabolic Insult and Enamel Growth Disruption

Dental wear limited observation of perikymata on a large number of dental casts examined for this study. As a result, a total of 16 enamel defects from 15 individuals were selected to estimate the duration of disruption events. Perikymata counts on closeup SEM images were counted to determine the number of perikymata within the occlusal wall of an enamel defect (duration of metabolic disruption) and the overall period of insult and recovery (all perikymata within the defect) were measured using the Measure and Label plugin in Image J (v. 2.0.0). An average of 9 days was assumed for perikymata formation in both the Chiribaya Baja and Rinconada Alta sample (following Reid and Dean 2006; Barrett and Mast 2016). The results of defect duration analysis were compared to the previously published metabolic disruption times for the Chiribaya Baja

		Rinconada Alta				Chiribaya Baja			
	Ν	Average	SD	Duration*	Ν	Average	SD	Duration*	
Number of Perikymata within defect	16	7.2	2.2	64.25 days	16	8.1	3.3	72.9 days	
Number of Perikymata within occlusal wall	15	2.9	1.28	26.4 days	16	2.9	0.77	26.1 days	

*Table 5.4.* Chart comparing average number of perikymata and average duration of metabolic insult for the Rinconada Alta and Chiribaya Baja sample.

\*Duration= perikymata\*9 (average period of perikymata formation)

sample (Barrett and Mast 2016); and are presented in Table 5.4. For the observation of

perikymata in the occlusal wall, one defect was missing observable occlusal wall

perikymata, so the number of recorded observations is listed as 15 (see Table 5.4).

When comparing defect duration between the two samples, the Rinconada Alta sample appears to have a slightly lower number of perikymata per defect. This results in a lower average duration of metabolic insult for the Rinconada Alta sample by 8 days. However, when observing the actual duration of the metabolic interruption by observing the perikymata within the defect's occlusal wall, the average number of perikymata is almost identical. This results in an average duration of metabolic disruption time of approximately 26 days for both the Rinconada Alta and Chiribaya Baja samples.

This means the recovery period or "catch-up growth" after an initial period of metabolic insult in the Rinconada Alta sample lasted for 37.9 days (64.25 days total minus 26.4 days) on average. For the Chiribaya Baja sample, the recovery period is approximately 46.8 days (72.9 days total minus 26.1 days). These results suggest that while stress events in both samples lasted for the same period of time, individuals in the Rinconada Alta sample have a slightly shorter recovery period after initial metabolic insult than individuals from Chiribaya Baja. This difference is likely to be biologically insignificant.

### 5.7. Analysis of Dental Health and Stature Between Sites

Visual assessment of dental pathology was performed following Buikstra and Ubelaker (1994). The results of the analysis for the current sample are presented below (Table 5.5). The number of carious lesions, antemortem tooth loss (AMTL), and active abscesses were recorded for all individuals in the Rinconada Alta sample. Females had the highest average number of carious lesions and AMTL (see Table 5.5). Males had slightly higher average number of dental abscesses, but notably fewer average carious lesions and teeth lost antemortem (Table 5.5). These results were compared to the

analysis of dental health performed by Murphy (2004) for Puruchuco-Huaquerones

(Table 5.6). Females from both Rinconada Alta and Puruchuco-Huaquerones appear to

have a higher prevalence of antemortem tooth loss and carious lesions than males from either site (Table 5.6).

	Ν	x Caries	SD	x AMTL	SD	x Abscess	SD
Female	10	2.4	2.319	6.8	5.903	1.0	2.079
Male	11	1.6	1.206	1.1	1.136	1.5	1.809
Subadult	5	1.8	0.837	0	—	0	
Total	26	1.8	1.728	3.1	4.715	1.4	1.835

*Table 5.5.* Table illustrating the average number of caries, teeth lost antemortem (ANTM), *and abscesses* for the Rinconada Alta sample. N= number of individuals per group.

	Rinconada Alta			Puruchuco-Huaquerones				
	Ν	x Caries	x AMTL	x Abscess	Ν	x Caries	х ANTM	x Abscess
Female	10	2.4	6.8	1.0	33	4.4	5.6	1.5
Male	11	1.6	1.1	1.5	55	3.1	3.5	2.0

*Table 5.6.* Table comparing averages for indicators of dental health between the current Rinconada Alta sample and Puruchuco-Huaquerones (Murphy 2004).

Adult stature is the result of cumulative growth outcomes and can reflect the level of stress experienced by an individual during growth and development (Buikstra and Beck 2006). Salter-Pedersen (2011) previously estimated adult stature at Rinconada Alta using formulae derived from Central American populations (Genovés 1967; del Angel & Cisneros 2004) as no population-specific formulae were available at the time of her dissertation.

Average stature for Rinconada Alta was 157.8 cm for males and 145.2 cm for females (Salter-Pedersen 2011, p.46). When the Del Angel and Cisneros (2004) formulae were used to calculate average stature for the *current* Rinconada Alta sample, the average stature was 158.5 cm for males and 144.7 cm for females. This is markedly similar to Salter-Pedersen's (2011) original stature results. Stature at Puruchuco-Huaquerones was reported to be 160.0 (-2.5 cm) for males and 150.0 (-2.5 cm) for females (Murphy 2004) using the Genovés (1967) equations. Genovés (1967) measured recumbent length of the cadavers in his sample and suggested that the results of his regression equations be reduced by 2.5 cm to accommodate for the difference between standing stature and recumbent length. Many publications, including Murphy (2004), which use the Genovés equations do not make that adjustment. With this subtraction, the average stature estimates at Puruchuco-Huaquerones are 147.5 cm for females and 157.5 cm for males (Table 5.7). When adjusted, the average stature between Rinconada Alta and Puruchuco-Huaquerones is very similar in regard to stature, although females from the current sample appear to be slightly shorter.

Due to the lack of population-specific formulae available at the time of Salter-Pedersen's (2011) dissertation, adult stature at Rinconada Alta was re-calculated (using measurements from the current sample) for males and females using stature formulae for physiological length of the femur, derived from the Lacerte and Slank (2018) formulae. These results (Table 5.8) are shown alongside the stature estimates for the Chiribaya Baja sample (Lacerte and Slank 2018). The average statures for Chiribaya Baja, which were

	Rinconada Alta			Puruchuco-Huaquerones			
	N	Average Stature	Stature Range	Ν	Average Stature	Stature Range	
Female	9	144.7	139.2-153.3	36	150.0*	137.6-159.2	
Male	10	158.5	156.6-164.0	57	160.0 *	151.7-168.7	

*Table 5.7.* Comparison of average male/female stature between the current study and Puruchuco-*Huaquerones* using the Del Angel and Cisneros (2004) stature formulae. All measurements are in centimeters (cm). Stature ranges are included to make up for Murphy's (2004) use of the Genovés (1967) formulae on the PH sample. \*Stature results as reported by Murphy (2004). With the -2.5 cm correction added, male stature= 157.5 cm; female stature=

<sup>\*</sup>Stature results as reported by Murphy (2004). With the -2.5 cm correction added, male stature= 157.5 cm; female stature= 147.5 cm

		Rinconada Alta				
	N	Average Stature	SD	N	Average Stature	SD
Female	9	145.6	4.7	9	148.2	4.2
Male	10	156.2	3.9	10	156.3	5.7

*Table 5.8.* Comparison of average male/female stature between the current study and Chiribaya Baja using the Lacerte and Slank (2018) stature formulae. All measurements are in centimeters (cm).

estimated using Chiribaya-specific regression formulae were 156.3 cm for males and 148.2 cm for females using the physiological length of the femur (Lacerte and Slank 2018). Since the original data from Murphy (2004) regarding limb length was not available, direct comparison between stature estimates by recalculation using the same formulae was not possible, hence the use of multiple stature formulae for this analysis (Tables 5.7 and 5.8). However, when we consider the adjusted formulae for Puruchuco-Huaquerones, the statures across all three sites are markedly similar.

### 5.8. Analysis of Childhood Stress Events Between Sites

One goal of this thesis is to test the assertion by previous researchers that the population at Rinconada Alta was under more stress than the population at the nearby site of Puruchuco-Huaquerones (Salter-Pedersen 2011). While microscopic analysis of LEH was not conducted at Puruchuco-Huaquerones, Murphy (2004) does provide unpaired LEH counts for all teeth observed at the site.

For the purposes of direct comparison, total number of visual LEH observations per individual was calculated for Rinconada Alta using the same method of using unpaired LEH counts outlined by Murphy (2004). This included all anterior and posterior adult teeth, although only anterior teeth were included in the SEM LEH analysis portion of this thesis. Murphy (2004) notes, "The mean number of enamel defects per individual on all teeth is 8.1 with a standard deviation of 6.6. Numerous individuals possessed multiple enamel defects and these high numbers probably reflect the same physiological perturbation affecting different teeth" (p. 96). In comparison, the unpaired average number of LEH per individual using the Rinconada Alta sample (calculated per Murphy 2004) from this thesis was 11.0 with a standard deviation of 5.9. The difference between unpaired average number of LEH ( $\bar{x}$  11.0) and paired LEH ( $\bar{x}$  2.2) highlights the importance of pairing defects across teeth to prevent over-estimation. This reflects the finding by Salter-Pedersen (2011), who notes the frequency of hypoplastic defects is greater at Rinconada Alta than at Puruchuco-Huaquerones. The higher number of unpaired LEH at Rinconada Alta does not suggest on its own, however, that the population at Rinconada Alta was under more stress than its sister site (cf. Wood et al. 1992).

This difference in methodology between studies highlights the importance of standardizing LEH observations across multiple sites and research projects. Of note is the fact that Murphy (2004) included both anterior and posterior teeth in her analysis of LEH, while both this present thesis and Salter-Pedersen (2011) only examined anterior teeth. Despite this, the number of teeth included in the Puruchuco-Huaquerones sample is actually less (Murphy 2004). Since some teeth are known to be more developmentally sensitive (Goodman and Rose 1990; Berbesque and Hoover 2018) to enamel disruption, selecting particular teeth for LEH analysis and excluding others may impact the number of recorded observations for a particular sample. An in-depth review of methodology and its implications for interfering differences in stress between the two sites will be included in the following chapter (Chapter 6).

### CHAPTER 6: DISCUSSION

### 6.1. Introduction

This chapter will focus on interpreting and discussing the experience of childhood stress at Rinconada Alta, with a focus on recent critiques of the "weaning hypothesis" in bioarchaeology. It will also revisit the suggestion by previous researchers that the population at Rinconada Alta was under more stress than the nearby site of Puruchuco-Huaquerones during the period of Inca occupation. Despite the differences in sample size, this thesis suggests the Rinconada Alta sample study and the Puruchuco-Huaquerones samples are similar in many regards. Additionally, the comparison between LEH duration in the Rinconada Alta sample and Chiribaya will be discussed, with a focus on suggestions for expanding and standardizing dental studies on archaeological populations from the Andean region.

### 6.2. Differences in LEH Frequency by Sex at Rinconada Alta

Previous research (Goodman et al. 1987; King et al. 2005) has suggested that in some cultures with existing male bias, females have a greater number of total LEH due to preferential treatment of male infants and children. Murphy (2004) provides LEH counts by sex for the sample from Puruchuco-Huaquerones. Murphy (2004) reports that adult males from the Puruchuco-Huaquerones sample had more unpaired hypoplastic defects (57.3%) than females (39.3%) but that these results are not statistically significant (p=0.09). It should be noted that these LEH percentages represent the total number of unpaired LEH observations from adult males, females, and adolescents in the sample.

When comparing the mean number of defects between adult males, females, and adolescents in the sample, the results of the Kruskal-Wallis one-way ANOVA on ranks

suggest there are no statistically significant differences between average number of LEH per subgroup for the sample. When examining both adults and adolescents from Rinconada Alta, adolescents (n=5) have a slightly higher average number of paired stress events per individual ( $\bar{x}$  2.4) than adult males ( $\bar{x}$  2.2). This is because the subadult group is the only group to have one individual (RA 1041A) with 5 separate observable stress events.

The adolescents were not sexed, making it impossible to evaluate the sex ratio of the individuals included in the adolescent group. Although it may be tempting to interpret the lower number of LEH in adults as evidence that individuals who lived to adulthood experienced slightly fewer stress events than those that died as adolescents, the sample size in this instance, and the Osteological paradox, warn against such a straightforward interpretation (Wood et al. 1992). Labial wear of the enamel surface, and loss of crown height in adult individuals in the sample may mean early-forming defects and surface enamel wear have slightly reduced the number of observable defects in the adult portion of the current sample.

### 6.3. Differences in Dental Health and Stature Between Sites

Published research by Williams and Murphy (2013) suggests male and female individuals at Puruchuco-Huaquerones varied slightly in their dietary access to protein and overall dental health, with females having more carious lesions and antemortem tooth loss overall. Males at Puruchuco-Huaquerones also appear to be more affected by periosteal infections, leading Williams and Murphy (2013) to conclude that increased labor demands (*m'ita*) may have led to delayed healing due and insufficient caloric intake in males during community-wide food shortages. Returning to the issue of temporal context, there is some question as to whether the sample Marsteller (2015) examined is entirely composed of Late Intermediate period Yschma burials as she claims, or whether there is admixture with later Yschma burials dating to the Late Horizon and period of Inca occupation. Despite this, research by Marsteller et al. (2016) suggests that the diet at Rinconada Alta followed the horizontal model of economic specialization first suggested by Rostworowski (1989; 2005). This means that despite close proximity to the ocean and marine resources, Rinconada Alta was a largely agricultural community, with some evidence of supplemental marine consumption, likely through trade with sites directly on the coast (Marsteller 2015; Marsteller et al. 2016). Additionally, isotopic analysis ( $\delta^{13}C_{col[VPDB]}, \delta^{15}N_{col[AIR]},$  $\delta^{13}C_{ap[VPDB})$  performed by Marsteller (2015) for her dissertation research suggests that the population at the site remained local.

When comparing dental health at Rinconada Alta using the current sample and Puruchuco-Huaquerones (Murphy 2004), it is apparent that the sample at Puruchuco-Huaquerones has slightly higher averages for carious lesions, antemortem tooth loss, and dental abscesses, possibly due to the larger size of the sample at Puruchuco-Huaquerones (Williams and Murphy 2013). Despite this, the similar trends in dental health highlighted by this current thesis research support the earlier assertion made by Williams and Murphy (2013) that the pattern of antemortem tooth loss and carious lesions in the Puruchuco-Huaquerones sample most closely resembles Salter-Pedersen's (2011) analysis of dental health for the Late Horizon period at Rinconada Alta. Adult females in the current Rinconada Alta sample have a higher average number of caries and antemortem tooth loss when compared to males, which is also seen in the sample from PuruchucoHuaquerones (Williams and Murphy 2013). However, adult males at both sites have a greater average number of abscesses than females.

One explanation for the discrepancy between male and female dental health at Rinconada Alta is the oral processing of maize for the production of fermented beverages like *chicha* (Jennings 2005; Lanfranco and Eggers 2012). This fermented beverage has ritual and cultural significance to many cultures throughout Andean prehistory (Valdez 2006). Lanfranco and Eggers (2012) note that in many modern-day cultures throughout the Andean and Amazonian regions of South America women and children are known to chew plants in the preparation of these fermented beverages. This difference would likely be isotopically undetectable, as the *chicha* paste is removed from the mouth and not ingested. In support of this, there is no significant difference in C<sub>4</sub> plant (*Zea mays* belongs to this group) consumption by sex at either Puruchuco-Huaquerones or Rinconada Alta (Williams 2005; Marsteller 2015). However, the mastication of stoneground maize by females in these populations could potentially lead to a more acidic oral environment, and frequent abrasion by particulates has the potential to cause an increase in carious lesions and other oral pathology.

For coastal populations in the Late Intermediate Period, Lanfranco and Eggers (2017) note that coca consumption between males and females appears to have been relatively equal. If coca chewing were the only cultural factor impacting dental health at Rinconada Alta, we might expect to see very similar patterns of antemortem, posterior tooth loss commonly associated with this practice (Indriati and Buikstra 2001; Lanfranco and Eggers 2017). Additionally, Salter-Pedersen (2011) notes that only a few individuals examined at Rinconada Alta had a pattern of antemortem tooth loss consistent with

regular coca chewing. Altogether, the difference between antemortem tooth loss, dental caries, and active abscesses between males and females at Rinconada Alta and Puruchuco-Huaquerones (Murphy 2004; Williams and Murphy 2013) suggest another factor is involved.

One possibility is that increased differences in health by sex between Rinconada Alta and Puruchuco-Huaquerones during the Late Horizon period could reflect an increase in labor demands on the *ayllu* (including chicha production) under Inca rule. Alternatively, the pattern of dental health observed at both sites could also reflect a continuation of previous activities prior to Inca conquest. For example, there is ample evidence for chicha production throughout the Andean region prior to the Late Horizon (Hayashida 2009), and the pattern of oral health observed at both sites might suggest a continuity of this practice throughout several periods of occupation on the Central coast.

Research also suggests that pregnancy may impact female oral health in both modern and archaeological populations (Watson et al. 2010; Lukacs 2011). Changes in salivary pH can lead to gingival inflammation during pregnancy and possibly decrease oral alkalinity, potentially leading to the development of caries (Watson et al. 2010). Additionally, Lukacs (2011) found that some modern differences in oral health between males and females are linked to variation in diet and cultural behaviors (i.e.: religious fasting, cultural, gender-based preferential access to food) between adult men and women of childbearing age, which can place women at increased risk. Bioarchaeological analyses have found numerous sex differences in the dental health of past populations; these have typically been associated with the rise of agricultural subsistence in societies around the world (Watson et al. 2010). In a sample of Early agriculturalists from La Playa, Mexico (1,600 BC- AD 200), Watson et al. (2010) found a significant difference in antemortem tooth loss between males and females at the site, but no difference in the rate of dental caries.

However, this is not the case for the present study and the results from Puruchuco-Huaquerones (Murphy 2004), which suggest carious lesions and antemortem tooth loss were greater for females than males at both sites. It is likely that the sex differences in oral health at Rinconada Alta and Puruchuco-Huaquerones reflect comorbid cultural and physiological factors and are unlikely to be the result of a single behavioral or physiological etiology.

While average stature (using formulae from Lacerte and Slank 2018) for females at Rinconada Alta is lower than the site of Chiribaya Baja (Lacerte and Slank 2018), it is also lower than average female stature (149.8 cm) reported elsewhere throughout the Andean region (Pomeroy and Stock 2011). It should be noted that both Salter-Pedersen (2011) and Murphy (2004) employed stature formulae for Mesoamerican populations (Del Angel and Cisneros 2004; Genovés 1967) as no population-specific regression formulae were available at the time of their dissertation research. However, when average stature was re-assessed for the Rinconada Alta sample from this thesis using the Chiribaya-specific formulae (derived from Raxter et al. 2006) for maximum femoral length (Lacerte and Slank 2018), average stature for Rinconada Alta was 156.2 cm for males (n=10) and 145.6 cm for females (n=9). Translated, this means there is an approximately 3.0 cm difference between Rinconada Alta and the Chiribaya Baja sample.

For Puruchuco-Huaquerones, despite the fact that Murphy (2004) employed the unrevised Genovés (1967) formulae, we see that the average statures are similar to those reported for Rinconada Alta when we include the 2.5 cm adjustment. There is also an appreciable overlap in the range of statures between Puruchuco-Huaquerones (Murphy 2004) and Rinconada Alta, suggesting that the minor variation in average statures between the two sites is based on difference in formulae being used and on individual variation within the samples (e.g.: sample size). Therefore, these differences in stature are unlikely to be largely biologically meaningful and do not seem to suggest drastic differences in overall health outcomes, at least in regard to adult stature.

Although isotopic estimates of age-at-weaning at Puruchuco-Huaquerones align with age-at-increased incidence of childhood stress at Rinconada Alta (Williams 2005; Marsteller 2015), weaning stress is only one factor that impacts an individual's overall experience of stress during growth and development. Average stature at Puruchuco-Huaquerones is also very similar to Rinconada Alta and Chiribaya Baja (Murphy 2004). While Puruchuco-Huaquerones has a smaller number of unpaired LEH than Rinconada Alta, similarities in average stature could suggest the population at Rinconada Alta was not under increased stress compared to its sister site.

In the case of this study, the sample size is too small to make an internal comparison of average stature between possible Late Intermediate period burials and those decidedly from Inca sectors of the cemetery. If we accept the working assumption that the majority of burials included in this thesis sample date to the period after the Inca conquest, then these stature results potentially suggest that growth outcomes at Rinconada Alta after ~AD 1470 remained similar to those for pre-conquest coastal populations (e.g.: Chiribaya Baja).

## 6.4. Comparison of Enamel Hypoplastic Defect Duration

The only geographically similar sample with which to compare the duration of metabolic disruption in the Rinconada Alta sample is derived from the Chiribaya population of Southern, coastal Peru (Barrett and Mast 2016). Unlike the sample from the current study, the burials from Chiribaya Baja date between AD 900-1300 and do not contain any remains from the Inca Period (Late Horizon). The length of stress episode duration between Rinconada Alta and Chiribaya Baja is notably similar (~26 days), but total insult recovery time in the Rinconada Alta sample is shorter by about 10 days.

It is possible that this time difference reflects a shorter period of recovery for the Rinconada Alta sample, suggesting they were slightly more resilient than the Chiribaya on the Southern coast. However, given the small sample size for both groups, and heavy dental wear observed in both samples (Barrett and Mast 2016), a difference of 10 days is not likely to be biologically significant in terms of overall population stress. For example, Barrett and Mast (2016) compare the duration of metabolic insult in the Chiribaya to an Inupiat sample from Point Hope, Alaska (Guatelli-Steinberg et al. 2004). In contrast to the current study, stress episode duration in the Inupiat was 70.2 days (Guatelli-Steinberg et al. 2004; Barrett and Mast 2016), much longer than stress episode duration for either Rinconada Alta (26.4 days) or Chiribaya Baja (26.1 days). Total recovery time from initial metabolic insult for the Point Hope Inupiat was approximately 120.6 days (Guatelli-Steinberg et al. 2004; Barrett and Mast 2016). The Point Hope Inupiat were a population under harsh arctic conditions meaning diet, total daylight hours, and access to resources were all limited at various times of the year and therefore this group represents a population under known, extreme stress (Guatelli-Steinberg et al. 2004).

Although Chiribaya Baja and Rinconada Alta were both coastal settlements located close to the Pacific Ocean (Nystrom and Malcolm 2010; Salter-Pedersen 2011), occupants focused on agricultural, rather than marine subsistence strategies (Tomczak 2003; Marsteller et al. 2016). Given the similar duration of enamel growth disruption between Rinconada Alta and Chiribaya Baja, it could be suggested that populations at Rinconada Alta did not undergo a significant increase in stress due to Inca conquest or were effectively able to buffer their children against the negative impact. Additional studies are needed to examine the difference in childhood stress episode duration between other pre-Columbian coastal and mid-altitude sites throughout the Andean region. These would provide a more nuanced and complete picture of childhood stress before and after Inca conquest.

# 6.5. Enamel Hypoplastic Defects and the "Weaning Hypothesis"

Several notable papers have critiqued the examination of LEH and its relationship to weaning stress in archaeological populations (e.g. Katzenberg et al. 1996). The primary argument in favor of this hypothesis is that infants and children are more susceptible to mortality and morbidity during the weaning period. The immune system of infants is not yet fully developed, and they often struggle to produce and secrete a variety of antibodies; in addition to having immature, underdeveloped respiratory and digestive systems (Cacho and Lawrence 2017). Breastmilk provides some postpartum immunity via antimicrobial proteins and peptides. This buffering effect decreases as the infant ages, as breastmilk loses some of these antibodies in favor of increasing caloric and nutrient content to aid in infant growth (Cacho and Lawrence 2017). The buffering effect ends with the cessation of breast feeding.

The traditional "weaning hypothesis" argues that weaning increases a child's frailty due to the loss of maternal immune factors found in breastmilk (Mays et al. 2017). This condition, in conjunction with the introduction of solid foods and potential accompanying infectious agents, is believed to weaken the infant's immune system, leading to elevated stress and the formation of linear enamel defects (e.g.: Katzenberg et al. 1996; Mays et al. 2017). However, critiques of this hypothesis suggest that the cessation of breastfeeding occurs over a prolonged period of time, so the total transition to solid foods at the end of the weaning period is unlikely to cause the "spike" in stress necessary to interrupt enamel formation (Katzenberg 1996; Mays et al. 2017). Therefore, some bioarchaeological literature (e.g.: Ritzman et al. 2008) has critiqued the usefulness of enamel defects in determining culturally significant events like weaning due to their non-specific nature and the variable sensitivity of enamel to metabolic disruption. Ritzman et al. 2008 and suggest that methodological differences between formulae to calculate defect age estimates create alternate explanations for this clustering of childhood stress events in their study sample.

Ritzman et al. (2008) examined LEH in a sample of 63 teeth from the ancient Nubian site of Semna South (located in modern-day Sudan). The goal of this research was two-fold. The first was to estimate the number of childhood stress events in the study sample, and the second was to compare the regression formulae (Goodman and Rose 1990) to the decile chart method of LEH age estimation developed by Reid and Dean (2006) from the South African sample (Ritzman et al. 2008). The authors found a significant difference in the event age estimates produced by both methods. The Reid and Dean (2006) method produced average age estimates up to 1.5 years older than those produced by the Goodman and Rose (1990) formulae, suggesting the pattern of enamel disruption at the site provided conflicting results based on the method of defect age estimation (Ritzman et al. 2008).

It should be noted that Ritzman et al. (2008) employed six-month intervals for the age categorization of enamel defects, as does this study. As discussed below (see Figure 6.2), the way defect ages are categorized can impact the "clustering" of childhood stress events. The authors observed an increase in LEH frequency in the sample around the ages of 4.5-5.0 using age estimates produced by the Reid and Dean (2006) decile method (Ritzman et al. 2008). As the age estimates produced using the Reid and Dean method (2006) show a "peak" in defect occurrence at a slightly older age than those produced by the Goodman and Rose (1990) method, the authors suggest that aquatic parasitic infection due to increased contact with contaminated water explains the clustering of stress events around this age range, rather than weaning stress alone. Ritzman et al. (2008) note that modern Sudanese children typically begin to swim and participate in water-related subsistence activities around the age of five, a practice that may have been present in their archaeological sample.

Despite issues surrounding the methods used to interpret the cause of childhood stress events, emerging research on the Developmental Origins of Health and Disease (DOHaD) hypothesis may help situate the examination of linear hypoplastic defects within bioarchaeologist's larger interpretation of the life course of past peoples (Armelagos et al. 2009). Research by Temple (2014) suggests a positive correlation between age-at-first enamel defect and later age-at-death in a population of Jomon period (4000-2300 BC) hunter-gatherers from the Japanese islands of Hokkaido and Honshu. While LEH is, by itself, a non-specific indicator of stress, when combined with other information about archaeological populations it has the potential to help bioarchaeologists evaluate larger population trends. More recent bioarchaeological studies (Temple 2014; Watts 2015; Mays et al. 2017) have begun to investigate this relationship in greater detail. Mays et al. (2017) suggests research on the "bioarchaeology of childhood" in the past ten years has greatly improved our understanding of adult health outcomes in archaeological populations. This "life course" approach, which situates childhood health/stress within the framework of overall health during an individual's lifetime, has only been implemented in bioanthropological studies since the mid-1990s (Mays et al. 2017).

Additionally, research by Watts (2015) has examined the impact of childhood stress on adult health in a post-medieval population from London, England. Watts (2015) found that early developmental perturbations during infancy and childhood did not appear to have a detrimental effect on adult longevity, although growth disruptions that occurred during adolescence or late childhood did significantly impact adult age-at-death in the sample. This is because metabolic insult is less disruptive if resolved early, while if the child is nearing the end of growth/development and still experiencing moderate stress, the effect on adult health will likely be more pronounced (Watts 2015). As a result, the examination of LEH and childhood stress in archaeological populations remains an important aspect of dental bioarchaeology, especially when other, supporting data is included to situate it within the life course of an individual.

## 6.6. Comparison of Methods for Defect Age Estimation

This thesis purposely estimated the frequency of enamel defects in the Rinconada Alta sample using two separate regression formulae methods (Goodman and Rose 1990; Cares-Henriquez and Oxenham 2019) to test for possible differences in peak defect age estimates. Several previous studies (Martin et al. 2008; Ritzman et al. 2008) have suggested that the Goodman and Rose (1990) formulae, which assume a linear pattern of enamel growth, do not account for cuspal enamel formation. The Reid and Dean 2006 method, which used unworn teeth from a sample of Northern Europeans and South Africans, uses "decile charts" to estimate the age at which a defect occurred. As previously discussed, the dental wear on a large portion of the sample made formulaebased age estimation methods more favourable as they do not require a large sample of unworn anterior teeth. The limitations and comparison between the two methods used on the Rinconada Alta sample are discussed below.

The Goodman and Rose (1990) formulae were originally based on tooth development times from the 1940s (Massler et al. 1941) and crown heights measured by Torsten Swärdstedt (1966) from a medieval Swedish population. One major criticism of this method is that it does not account for variable cuspal enamel formation time as the regression formulae are linear, and do not account for variable growth rates around this initial formation period or the "hidden" enamel that is unobservable on the enamel surface (Goodman and Song 1999). The way in which the timing of this early depositional stage is calculated can impact age estimates for enamel defects on the enamel surface (Goodman and Song 1999; Reid et al. 2008). Prior to the publication of research by Reid and Dean (2006), Goodman and Song (1999) address the some of the shortcomings of the Goodman and Rose (1990) formulae and suggest that when cuspal enamel formation was accounted for in their study sample, the biological difference equaled a period of less than 3 months when appositional cuspal enamel formation is included in the defect age estimates (Goodman and Song 1999). Goodman and Song



*Figure 6.1.* SEM image of right maxillary canine (RA 1119A) from Rinconada Alta sample highlighting unilateral loss of crown height on only one side of the tooth.

(1999) also strongly suggest using population-specific average crown heights to aid in the age estimation of enamel defects, due to variation in crown height between different human populations.

When an archaeological sample has heavily worn dentition, it can be difficult to find unworn teeth to implement the Reid and Dean (2006) decile method. While teeth with >30% wear were excluded from the current sample, all teeth cast for this thesis research were worn to some degree. Additionally, not all dental wear occurs evenly, with the mesial or distal side potentially being more heavily worn depending on dental malocclusion or occupation-specific activities that alter the appearance of the teeth. Therefore, while estimating remaining crown height is often a good measure of observable surface enamel, this can be complicated by atypical or uneven wear patterns

on the teeth themselves. A mild example from this current study (Figure 6.1) illustrates this. The cusp of the tooth is still visible, but one side of the tooth is more heavily worn than the other.

More recently, research has been proposed to help resolve some of the issues related to studying worn dentition in archaeological populations (Cares-Henriquez and Oxenham 2017; 2019). An LEH calculator, based on the Cares-Henriquez and Oxenham (2019) formulae is available for download from: https://www.lehtools.com/ and was used here to compare age estimates to the Goodman and Rose (1990) formulae. It should be noted, however, that this calculator still employs the original Reid and Dean (2006) South African and Northern European data.

When Cares-Henriquez and Oxenham (2019) compared the Goodman and Rose (1990) linear regression method to their suggested exponential regression method of defect age estimation, they found that the linear age estimates were, on average, 8 months younger than those produced by the exponential regression formulae. In the present study, the difference between the two age estimations is smaller. The age estimates for Rinconada Alta produced by the Cares-Henriquez and Oxenham (2019) exponential formulae are approximately 1.3 months younger than those produced by Goodman and Rose (1990), but the results from the two different methods are still statistically significantly different from each other at the alpha level 0.05 (p=0.007).

Despite this difference in formulae, when the number of defects per individual within a given age range (0.0-7.0) was assessed, both formulae indicate the greatest number of defects occur between the ages of 3.51-4.0 (see Chapter 5). Although none exists for Rinconada Alta, Williams (2005) does provide isotopic ( $\delta^{15}N$ ) information
about weaning ages from Puruchuco-Huaquerones. The age range of 3.51-4.00 for the sample from Rinconada Alta is consistent with estimated time of cessation of weaning reported by Williams (2005). While children older than four years had less  $\delta^{15}$ N enriched values than those under three years of age, Williams (2005, p.190) notes that due to the "…time lag between dietary change and isotopic change in bone…weaning at Puruchuco-Huaquerones was likely completed closer to three years of age".

While there is a statistically significant difference between the defect age estimates generated from the two formulae (Goodman and Rose 1990; Cares-Henriquez and Oxenham 2019), the average difference (1.3 months) between the two methods is likely to be biologically insignificant. One explanation for the lower difference in age between the two methods in this current study and the linear/exponential comparison performed by Cares-Henriquez and Oxenham (2019) is the extent of dental wear in the Rinconada Alta sample. Relatively few enamel defects are recorded between birth and 2.0 years using either method (see Chapter 5). As the crown of the teeth form from the cusp toward the cemento-enamel junction, it is possible that very early defects in the Rinconada Alta sample are being "lost" due to enamel surface wear and reduction in crown height.

Temple (2014) and King et al. (2005) also address dental wear in archaeological samples. Developmentally early defects around the cusp of the enamel have been previously shown to create the greatest difference in defect age estimates between linear and decile methods (Ritzman et al. 2008; Martin et al. 2008). Most of the observable defects in the Rinconada Alta sample occur on the middle third of the tooth crown (see Chapter 5). Goodman and Song (1999) note that the age estimates of developmentally older defects (closer to the CEJ) should be less affected by cuspal enamel formation times. Interestingly, there appears to be a difference in the age estimates of late-forming defects in the current sample between the Goodman and Rose (1990) and Cares-Henriquez (2019) formulae (Figure 6.2).

The slightly older age estimates produced by the Goodman and Rose (1990) formulae also appear to create a smaller, secondary "spike" in the number of individuals with LEH between the ages of 5.1-5.5 (see Figure 6.2). When compared to the age estimates for Cares-Henriquez and Oxenham (2019) there is no "spike" in the number of individuals with LEH around this age range; rather, the number of individuals with LEH per age range declines steadily after 4.0 years (see Chapter 5).

This "spike" around 5.0 years can also be observed in LEH age estimation charts produced by Dolphin (1999) for her dissertation research on two Postclassic Maya samples from Belize. Dolphin's (1999) thesis was selected as an example as she also used the Goodman and Rose (1990) formulae to estimate LEH age/frequency between her two samples. When Dolphin (1999) adjusted her chart to display one-year rather than halfyear age intervals, these two "peaks" disappeared, suggesting the method of determining



*Figure 6.2.* Graphs illustrating the difference between using half-year and one-year intervals to group LEH by age using the Goodman and Rose (1990) and Cares-Henriquez and Oxenham (2019) formulae.

enamel defect age categories can impact the interpretations of results. When the age ranges for the Rinconada Alta sample using both formulae are transformed from half-year to one-year age intervals, the "peak" on the second graph is similarly "smoothed" (Figure 6.2).

When these factors are considered, this suggests that the second "peak" observed using the Goodman and Rose (1990) sample in both the current study and Dolphin (1999) is likely related to how age estimates are calculated using the linear regression formulae (Goodman and Rose 1990) are "sorted" when placed into standard half-year intervals for LEH formation. If the reason for the second "peak" around 5.0-5.5 years was metabolic rather than methodological, we would expect to see a similar increase in number of individuals with LEH when sorting the Cares-Henriquez and Oxenham (2019) exponential regression formulae into half-year intervals. However, this lack of a "peak" using the alternate formulae suggests that the peak observed using the Goodman and Rose (1990) formulae is an "artifact" produced by the method of analysis and does not represent a real increase in the number of individuals with enamel hypoplastic lesions within that age range. This is consistent with Dolphin's (1999) suggestion that the method of analysis was the reason for the increase in defects around the age of five in her dataset for the Post-Classic Maya sites of Marco Gonzales and San Pedro.

However, there is still an observable difference in the late-forming LEH on the graph. While the Cares-Henriquez and Oxenham (2019) age estimates taper off between the ages of 5.0-6.0, the Goodman and Rose (1990) produce slightly older age estimates for the same late forming (<5.0 years of age) defects (see Figure 6.2). One possible explanation is the inclusion of dental wear and its effect on the age estimates for the

Cares-Henriquez and Oxenham (2019) formulae. Additionally, the exponential nature of the Cares-Henriquez and Oxenham (2019) formulae, which accounts for cuspal enamel formation, may be producing slightly younger defect ages overall than the linear regression formulae. Finally, slight differences between the two samples in regard to ageat-completion for crown formation may be aging the defects using the Goodman and Rose (1990) by a few months. Therefore, the average difference of 1.3 months (see Chapter 5) in defect age estimates between the two formulae may be particularly prominent in late-forming defects due to each method's assumptions about when crown development begins and ends.

This example highlights some of the shortcomings of using particular methods to estimate the age of defect occurrence on the enamel surface of teeth. While there has been one attempt to develop population-specific dental eruption/root completeness times from an archaeological sample from the Andean region (Gaither 2005), there is no published data on enamel formation times for South America specifically. Regardless of method used, the age estimates for LEH produced by current methods do not cover a broad range of populations. If population-specific enamel formation times have a subtle but significant effect on age estimates (Reid and Dean 2006), and if different methods produce different age estimates in the same sample (Ritzman et al. 2008; Martin et al. 2008) then future bioarchaeological dental studies need to develop and be based upon population-specific enamel formation times for archaeological populations in the Andean region.

### 6.7. Sample Limitations and Directions for Future Research

Despite the relatively small number of individuals examined for this thesis, it should be noted that the sample size of 26 individuals presented in this thesis is not inconsistent in size with other dental anthropological publications which use microscopic methods to examine LEH frequency and duration in historic and prehistoric populations (i.e. Guatelli-Steinberg et al. 2004; King et al. 2005; Temple 2014). Additionally, several precautions were taken to avoid sample bias. First, steps were taken to prevent biasing the sample towards young adult or adolescent individuals with less loss of crown height and labial enamel wear (see Chapter 4). Individuals with severe skeletal pathology were also excluded (see Chapter 4). The cross-site and cross-study comparisons presented in this thesis also provide secondary support that the smaller sample used in this thesis is still consistent with the larger Rinconada Alta sample as a whole. Salter-Pedersen (2011, p.32) examined 116 individuals from Rinconada Alta for her dissertation work, including several of the individuals included in this study. The consistency of average adult stature results between the current thesis and Salter-Pedersen's (2011) dissertation suggests the individuals in this study are more likely to be representative of the large collection of skeletal material (*material óseo*) recovered from the site. Cross-site average stature comparison with Puruchuco-Huaquerones and Chiribaya Baja also supports this conclusion by illustrating the similarities in stature between the current Rinconada Alta sample and other coastal sites from the Andean region (Murphy 2004; Lacerte and Slank 2018).

Studies from the Andean region are clearly underrepresented in bioarchaeological dental literature. While some bioarchaeological investigations from this region have attempted to match LEH across antimetric pairs of teeth (Andrushko 2006; Klaus and

Tam 2009), there is still a lack microscopic dental analyses from Peruvian archaeological samples (Klaus and Tam 2009). As illustrated in the current study, comparison of LEH counts between sites must often forgo the current standards in dental anthropology which recommend matching hypoplastic enamel defects across pairs of teeth whenever possible (Guatelli-Steinberg et al. 2004; Temple and Goodman 2014).

The lack of published data on enamel defect duration (perikymata within defect counts) in archaeological Andean samples highlights the need for more studies which employ both macroscopic (visual) and microscopic (SEM, etc.) LEH analysis. Given the implications of the Osteological Paradox (Wood et al. 1992) and emerging research surrounding the DOHaD hypothesis, future bioarcheological dental analysis in the Andean region should employ these methods to provide a more thorough analysis of childhood stress and create greater replicability and standardization of methodology between sites.

There is also a frustrating lack of population-specific standards for groups in this region. While previous research and this current study have highlighted differences between the common methods used to age enamel hypoplastic defects, all of the formulae evaluated for the purposes of this thesis are derived from European samples (Goodman and Rose 1900; Reid and Dean 2006; Cares-Henriquez and Oxenham 2019). While Reid and Dean (2006) suggest the variation in enamel formation times between populations is less than previously suggested, Gaither (2008, p. 99) does note in her thesis that the radiological and visual dental development of her South American samples has the potential to be advanced by as much as 1.5-2 years when compared to standard dental charts derived from "white" (Northern European) samples. Therefore, despite the

developmentally controlled nature of teeth across human populations (Reid and Dean 2006), using formulae based on crown heights from non-South American samples leaves much to be desired methodologically.

Suggestions for future directions for bioarchaeological dental research in the Andean region could include the development of population-specific enamel formation standards, and the evaluation of mean crown height in coastal and mid-altitude archaeological populations from this region to assist with the assessment of dental wear. Especially when using formulae where dental wear is taken into consideration (Cares-Henriquez and Oxenham 2019), variation in dental wear and rate of dental attrition between different populations (and diets) should be taken into consideration. Given the unique comorbid factors (i.e. coca chewing and chicha production) influencing oral health in the pre-Columbian Andean region, attention should be directed towards expanding regionally specific methods of dental age estimation for these populations.

### 6.8. Summary

While the dental evidence from Rinconada Alta and isotopic ( $\delta^{15}$ N) evidence for weaning age from Puruchuco-Huaquerones for weaning appears to be consistent, this does not necessarily mean that the increase in the number of childhood stress events around this age correlates directly to the cessation of breastfeeding. All three sites discussed in this thesis (Rinconada Alta, Puruchuco-Huaquerones, and Chiribaya Baja) were agricultural, despite their close proximity to the Pacific Ocean. There is some precedent from Chiribaya sites within the Osmore drainage (Martinson et al. 2003) that the residents of settlements located along coastal river valleys experienced increased parasitic load as a result of agricultural runoff from farther upstream, in addition to the habitual consumption of marine resources. Modern, clinical research suggests breastmilk has the potential to buffer the negative effects of some types of parasitic infection through the transmission of maternal antigens (Kutty 2014). However, this effect is reduced as the infant ages and the mother's breastmilk changes nutrient content before being lost at the end of the weaning period (Kutty 2014)

When additional osteometric data is included in the comparison between Puruchuco-Huaquerones, Rinconada Alta, and Chiribaya Baja, we see some slight differences, particularly in number of *unpaired* LEH. The duration of stress events at both Rinconada Alta and Chiribaya Baja suggests that both populations experienced similar duration and recovery periods for metabolic insult during childhood. Future research could compare perikymata counts within LEH defects at Puruchuco-Huaquerones to confirm whether there is a difference in the duration of childhood stress at the site. Despite the mixed temporality of the sample used in this study, it is possible that the slight differences observed between Rinconada Alta and Puruchuco-Huaquerones reflect differential health outcomes based on function between the two sites during the period of Inca occupation (P-H: administrative center vs. agriculturalists).

#### **CHAPTER 7: CONCLUSION**

The primary aim of this thesis was to re-evaluate childhood stress (linear enamel hypoplastic defects) at Rinconada Alta using more refined methods of dental analysis, including Scanning Electron Microscopy. The secondary goal was to compare these findings to other published data from the nearby site of Puruchuco-Huaquerones, a large sample of remains dating to after Inca conquest of the Central coast (Murphy 2004; Williams 2005; Williams and Murphy 2013). Third, this thesis tested methods of enamel defect age estimation to test whether there were any differences in age estimates for stress events produced by these formulae, as have been documented in other dental anthropological literature. This thesis also highlighted issues pertaining to the lack of accurate temporal association of burials used in previous studies of skeletal remains from Rinconada Alta, adding to our understanding of the site by re-testing some of Salter-Pedersen's (2011) previous conclusions, and providing new information

While this thesis included a few individuals who may date to both the Late Intermediate Period and Late Horizon (Inca period), the majority of the sample likely dated to after the Inca conquest. Previous research (Salter-Pedersen 2011) has suggested that the occupants of Rinconada Alta were under slightly more stress than those at the neighboring administrative center of Puruchuco-Huaquarones. However, when LEH lesions are re-evaluated using more refined techniques, this assumption is not fully supported by the dental evidence, particularly when examining paired vs. unpaired LEH counts for individuals at Rinconada Alta. I hope this example demonstrates the importance of standardizing the examination of LEH across Andean archaeological sites. Future research in this area could be expanded by introducing microscopic evaluation of hypoplastic enamel defects to more sites from the Andean region and by collating data on dental health and adult stature to examine larger trends across sites and time periods.

While age-at-weaning, adult stature, and levels of childhood stress at both Rinconada Alta and Puruchuco-Huaquerones appear to be similar, there are differences in the average number of unpaired LEH, suggesting there may be slight differences in overall population health between the sites. Some of these differences may be related to time period and subsistence activity/social status at Rinconada Alta, a similar conclusion reached by Salter-Pedersen (2011). Another issue is the lack of standardization between data sets, as Murphy (2004) examined the anterior and posterior teeth for LEH, while this study and Salter-Pedersen (2011) examined only the canines and central/lateral incisors. Despite the presence of some slight differences between the two sites, overall population stress during childhood at Rinconada Alta and Puruchuco-Huaquerones may be even more similar than previously suggested (Salter-Pedersen 2011).

While there were some differences in the recovery period for metabolic insults between Chiribaya Baja and Rinconada Alta, the duration of stress events at both sites was markedly similar, especially when compared to groups known to be under extreme environmental pressures like the Inupiat of Point Hope, Alaska (Guatelli-Steinberg et al. 2004). This suggests that at least compared to the Late Intermediate period site of Chiribaya Baja, the largely Inca-period sample at Rinconada Alta was not under markedly more stress post-conquest. Returning to the "Bioarchaeology of Imperialism" (Tung 2012), the findings from this thesis research support the conclusion that the Yschma territory was incorporated into the Inca empire without severe, negative disruption to population health on the Central coast (Eeckhout and Owens 2008). Therefore, when methodology can be standardized between sites and projects, the analysis of LEH and childhood stress remains a powerful tool for comparing and contrasting overall health between populations from the Andean region. Given the effect of childhood stress on adult health outcomes (cf. DOHaD), this important non-specific indicator of stress should not be overlooked, despite several critiques about its relationship to weaning age (Ritzman et al. 2008; Martin et al. 2008). Arguably, the importance of LEH lies not in its ability to estimate the timing of specific childhood stress events like weaning, but rather to measure the effect that stress events during childhood can have on age-at-death and other important adult health outcomes (Temple 2014).

Finally, the differences between the two regression-based formulae for estimating defect age highlight the need for further inclusion of diverse samples in this area of dental anthropological research. While there was a statistically significant difference between the two methods of defect age estimation, the average difference in days between the age estimates using both formulae was no more than 40.2 days (1.3 months), and when plotted with the data binned in one-year plots, the curves were essentially the same. This difference is smaller than those reported for other studies which compare LEH age estimates (Ritzman et al. 2008; Cares-Henriquez and Oxenham 2019). This somewhat supports the argument made by Martin et al. (2008) and Goodman and Song (1999) that small differences in age estimation methods are likely to be biologically insignificant. However, as discussed in the previous chapter (Chapter 6) the accuracy of the age estimates derived using either formulae cannot be adequately assessed without known enamel formation times for pre-Columbian teeth from South American populations.

To conclude, the individuals in the sample from Rinconada Alta did not appear to have had a significantly increased number of childhood stress events relative to the other coastal Andean sites used for this thesis comparison. While average female stature was *slightly* lower at Rinconada Alta than at Puruchuco-Huaquerones, the overlapping stature ranges for females at both sites indicates this is likely due to sample variation and size. The similar average number of LEH between adult males and females at Rinconada Alta also suggests that at least during early childhood, females at Rinconada Alta were not subject to significantly different stressors than their male counterparts, although they may be experiencing different patterns of oral health than males in later adulthood.

However, given the slightly lower female stature at Rinconada Alta relative to the Late Intermediate Period Chiribaya and Puruchuco-Huaquerones sample, population variation could be another possible explanation for this observed difference in stature. Despite this, the similarities in childhood stress duration between the pre-conquest coastal sample from Chiribaya Baja and Rinconada Alta (largely post-Inca conquest), supports the conclusion that overall population health at Rinconada Alta was not drastically altered after incorporation into the Inca Empire.

This thesis highlights the importance of interweaving a biocultural approach into the evaluation of dental enamel defects, taking a long view of the relationship between childhood stress and adult health. It also highlights the need for more refined methods of dental anthropological analysis in human remains from the Andean region. Future research in this area should expand our understanding of dental development in pre-Columbian populations from South America with the goal of combining this information about early childhood with adult health, age-at-death, and larger trends between different time periods and cultural groups/settlements.

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Site	Sector	CAJA	AF#	CODIGO	SEX	AÑOS	#Dientes			
RA	11	10	AF11902	217	F	38-49	12			
RA	IIA	5A	AF12163	288	F	30-40	10			
RA	IIA	10B	AF12124	720	F	20-25	10			
RA	IIAE	2	AF11941	873	F	30-40	8			
RA	IIAE	26		1019	F	25-35	8			
RA	IIAE	54	AF12014	1154	F	25-35	8			
RA	IIAE	62	AF12023	1169	F	18-25	10			
RA	IIAE	70	AF12034	1308	F	30-40	8			
RA	IIAE	75	AF12041	1353	F	40-55	10			
RA	IIAE	45	AF12153	1119A	F	20-35	10			
RA	IIA	198	AF15234	348	Adol.	1215	18			
RA	IIAE	60	AF12001	1115	Adol.	13-15	18			
RA	IIAE	43	AF12004	1117	Adol.	13-15	12			
RA	IIAE	44	AF12005	1118	Adol.	14-17	14			
RA	IIAE	32	AF11977	1041A	Adol.	13-15	8			
RA	П	29	AF11911	463	М	28-38	6			
RA	П	44	AF11934	563	М	20-25	16			
RA	П	50Y51	AF11954	574	М	30-40	8			
RA	IIA	3	AF12179	888	М	25-35	10			
RA	IV	14	AF12062	905	М	30-35	12			
RA	IIAE	27	AF11972	1024	М	20-30	10			
RA	IIAE	42	AF12003	1115.2	М	25-35	10			
RA	IIAE	67	AF12029	1198	М	35-49	6			
RA	1	16	AF11899	1135.2A	М	35-50	12			
RA	1	17	AF11900	1135.2B	М	20-35	14			
RA	IIAE	14	AF12097	967A	М	20-25	12			

# APPENDIX A—DENTAL CAST INVENTORY

RA#	Side	Position	Туре	Crown Height	% Missing Visual CEJ (mm)	) Α	kge (V)	Visual #			SEN	1 CEJ (m	nm)	SEM #	Age (	Goodn	nan an	d Rose	1990)	Age	(Cares-	Henri	quez 2	2018)
217	L	MAN	12	6.51	10 4.21	2.06		1	3.50					1	2.39					2.09			_	
217	R	MAN	12	6.215	10 4.2	2.07		1	3.60					 1	2.34					1.98				
288	L	MAX	C	8.702	10 3.36	3.9		1						 C										
288	R	MAX	C	7.82	10 3.16	4.03		1						 0										
348	L	MAX	C	9.129	5 4.67	3.08		1	1.86	2.87	3.90	6.98		 4	4.84	4.21	3.56	1.64		4.32	3.86	3.45	2.45	<b>;</b>
348	R	MAX	C	9.124	5 4.99 7.65	2.88	1.22	2	1.55	3.29	4.47	6.56		 4	5.03	3.94	3.20	1.90		4.47	3.68	3.23	2.57	/
463	R	MAX	11	5.15	10 4.24	2.58		1	2.20					 1	3.50					3.04				
463	L	MAX	11	5.602	15 4.44	2.48		1						 C						0.00				
463	L	MAX	C	9.348	10 4.51	3.18		1	1.53	2.21	3.60			 3	5.04	4.62	3.75			4.49	4.18	3.60		
463	R	MAX	C	6.904	20 3.11	4.06		1	1.19	1.91	2.79	3.52		 4	5.26	4.81	4.26	3.80		4.54	4.14	3.69	3.36	i
563	R	MAX	C	9.424	10 2.58	4.39		1	2.46	3.93	6.30			 3	4.46	3.55	2.06			4.07	3.48	2.70		
563	L	MAX	C	8.263	5 3.17 6.4	4.02	2	2	1.49	2.64	4.69			 3	5.07	4.35	3.07			4.42	3.84	2.99		
563	R	MAX	11	7.78	5			0	1.75					 1	3.70					3.72				
563	L	MAX	11	8.277	5			0	1.85					 1	3.66					3.73				
563	L	MAN	C	8.359	10 2.86 4.27 5.4	4 4.82	3.99 3.3	3	1.66	2.90	3.91			 3	5.53	4.80	4.20			4.82	3.99	3.42		
563	R	MAN	C	9.984	5 3.69 5.07	4.33	3.52	2	2.08	3.39	4.47			 3	5.27	4.51	3.87			4.76	4.03	3.51		
574	R	MAN	12	6.479	10 4.01	2.16		1	2.18					 1	3.00					2.72				_
574	L	MAN	12	4.446	10 4.04	2.14		1	2.55					 1	2.83					2.00				
574	R	MAN	C	7.391	10 4.91	3.61		1						 C										
574	L	MAN	C	6.556	10 4.31	3.97		1						 C										
720	L	MAN	C	9.684	10 4.28	3.98		1	2.04	2.54	3.13	4.26		 4	5.30	5.01	4.66	4.00		4.75	4.44	4.11	3.55	i
720	R	MAN	C	10.372	10 4.75	3.71		1	1.97	2.56	3.53	4.15		 4	5.34	4.99	4.42	4.06		4.87	4.53	4.03	3.73	1
873	L	MAN	C	8.639	10 3.29 6.54	4.57	2.65	2	5.65	ļ	<u> </u>			 1	3.18		<u> </u>			2.70				4
873	R	MAN	C	8	10 3.43 6.28	4.48	2.81	2	2.80	5.36				 2	4.85	3.35				3.98	2.65			
873	L	MAN	12	7.751	10 2.54	2.83		1	1.41	2.49	4.44			 3	3.41	2.96	2.15			3.32	2.77	2.00		4
873	R	MAN	12	7.586	10 3.5 6.17	2.39	1.16	2	1.58	2.47	4.39			 3	3.34	2.97	2.17			3.21	2.75	1.98		4
873	R	MAX	C	7.092	20 3.98	3.51		1	1.53	3.25				 2	5.04	3.97				4.37	3.51			
873	L	MAX	C	7.784	20 3.3	3.94		1	1.42	3.23				 2	5.11	3.98				4.50	3.65			
888	L	MAX	C	8.328	10 3.93	3.54		1	4.18					 1	3.39					3.47				_
888	R	MAX	C	8.003	10 3.66	3.71		1	3.88					 1	3.58					3.43				_
905	L	MAX	C	8.712	10 3.67	3.71		1	3.95	5.32				 2	3.53	2.68				3.36	2.86			
905	R	MAX	C	8.426	10 3.69	3.69		1	3.80	5.44				 2	3.63	2.60				3.36	2.77			
1019	L	MAN	C	8.472	10 3.95	4.18		1	1.43	6.26				 2	3.34	1.12				5.00	2.43			
1019	R	MAN	C	6.188	20 4.07	4.11		1	1.50	4.72				 2	3.31	1.83				4.72	2.62			
1024	R	MAX	C	8.661	10 4.71	3.06		1	2.78	4.07	5.55			 3	4.26	3.46	2.53			3.83	3.30	2.78		
1024	L	MAX	C	9.117	10 4.95	2.91		1	2.23					 1	4.61					4.10				
1115	L	MAN	C	10.156	5 2.9 3.97	4.79	4.17	2	2.27	3.36	5.04			 3	5.23	4.63	3.69			4.60	3.98	3.19		
1115	R	MAN	C	9.205	5 1.95 3.64	5.35		2	1.82	2.83	4.50			 3	5.49	4.92	3.99			4.76	4.11	3.22		
1115	L	MAX	11	10.76	5 4.58	2.42		1	3.49					 1	2.91					3.19				
1115	R	MAX	11	13.641	5 3.57	2.88		1	3.11					 1	3.09					3.55				_
1115	L	MAX	C	9.094	5 1.82 3.98 5.8	85 4.86	3.51 2.3	3	1.49	2.00	2.73	3.34	4.40	 5	5.07	4.75	4.29	3.91	3.25	4.45	4.19	3.85	3.58	3.17
1115	R	MAX	C	9.209	5 3.31 4.87	3.93	2.96	2	1.58	2.05	2.63	3.48	4.17	 5	5.01	4.72	4.35	3.83	3.39	4.42	4.18	3.91	3.55	3.27
1115.2	L	MAX	11	6.711	20 3.92	2.72		1	1.87	2.86				 2	3.65	3.20				3.61	3.04			
1115.2	R	MAX	11	6.774	20			0	1.59	2.83				 2	3.78	3.21				3.80	3.07			_
1117	L	MAX	C	11.018	10 2.54	4.41		1	2.47					 1	4.46					4.23				
1117	R	MAX	C	13.298	10 3.13	4.04		1	3.63					 1	3.73					4.03				
1118	L	MAN	12	8.392	10			0	4.60					 0	0.00									
1118	ĸ	MAN	12	7.254	10	0.07		0	1.68					 1	3.23					3.11				+
1154	ĸ	MAX	11	6.948	20 2.71	3.27		1	1.19					 1	3.96					4.09				
1154	L .	IVIAX	11	6.7	20 2.39	5.41		1	1.20	2.24					3.93	2.00				4.01	2 70			+
1169	L	MAX	11	/.411	10 1 02	2.02		0	1.40	3.31	2.00	-	-	 2	3.86	3.00	2 72		-	3.90	2.78	2.00		+
1109	к	IVIAX	11	5.1 7 220	10 1.93	3.02		1	1.42	2.20	5.90			 3	2.85	3.4/	2.73			3.9/ 2.75	5.47	2.00		+
1198	L	IVIAX		/.338	10 5.7	2.44		1	4.//						3.02					2./3		_		+
1198	ĸ	IVIAX MAX		0.0/1 E 020	20 0.12	2.0		1	J.01						2.50					2.00		_		+
1200	ĸ	IVIAX MAY		5.938 6.100	20 2 06	2 52		1	2 02					 1	2 54					2 02		_		+
1308	L	IVIAX MAN		7 601	15 1 02 1 24	3.33	2 25	1	3.95	1 70	3.00	1 05			3.54	2 22	262	214		2.95	1 74	3 00	3 ).	,
1000	L	IVIAN		1.001	10/ 2.24	4.0	3.02	2	1.18	2.70	3.00	4.05		 1	3.40	3.22	2.02	2.14		J.13 A 67	4./4	2.00	3.21	+
1000	к	IVIAN		E 702	2.54 2.24	4.79	3.70	2	1.44	2.02	4.14			 3	2.54	2.19	2.10			9.07	2.00	2.75		+
1000	L	IVIAX MAX	11	5./02	20 3.3	5 2 1 /	2.56	1	1.52	2.33				 1	3.01	3.35				3.00	2.30	_		+
10/11	K D	IVIAX MAY	r	5./4Z	20 2.99 4.26	1 0/	2.30	1	2.07	2.73	4.06	5.62	5 92	 	J.01	J.20	3 /17	2 /10	2.26	J.06	4.07	3 70	2.21	1 3 17
1041A	N	IVIAA MAX	~	11.301	5 6 5 2	1.74		1	2.07	1.51	5.00	3.05	3.30	 1	4.71	2 17	2.4/	2.40	2.20	4.41	4.07	3.70	3.21	. 3.12
1041A 1110A	L	IVIAX MAX		11.30	5 6 51	1.92		1	3.15	4.01	5.08			 1	+.U3	3.12	2.03			4.00	3.32	3.3/		+
1113A 1110A	L 0	MAY	~	11.720	5 5.51	2.00		1	1 20	2 2 2 2	5.09			 1	5.29	3.40	2.32			1.10	3.07	3.11		+
1119A 1110A	K D	IVIAX MAY	11	9.02/	5 4 52	2.71		1	1.50	3.33	J.12			 1	3 03	3.91	2.00			4.00	3.09	3.03		+
1119A	N 1	MAY	11	8,656	5 4 24	2.45		1	2.47	3.46	-			 2	3.02	2 92	-			3 11	2 87	-		+
076A	L D	MAY		Q 1Q4	5 6 21	2.30		1	457	5.40	-			 1	3.13	2.33	-			2 02	2.07	-		+
076A	N 1	MAY	~	6 221	20 5 22	2.12		1	3 7.9	4.00	-			 1	3.14	3 //	-			2.52	2 62	-		+
076A	1	MAN	~	7 525	10 6 42	1 00		1	1 NF		1			 1	3.04	5.44	1			3 1/	2.03	-		+
976A	L R	MAN		7.50	15 6 56	1.55		1	4.00		-			 1	3.47	-	-			2 90		-		+
1135 2A	<u>к</u> 1	MAY	r	0	10 3 87	3 58		1	3.08	3 28	-			 1	4 08	3 95	-			3.75	3.67	-		+
1135.24	D	MAY	~	7 274	15 2 77	5.50 NA 5		1	2.00	3.20	1			 1	4.00	4 00	1			3.65	3.57	-		+
1135.2A	N 1	MAY	~	0.0/1	5 2 07	A 1A		1	2.00	5 22	-			 1	4.21 4.71	2 72	-			J.00 4.2⊑	3.03	-		+
1135.2D	L R	MAY		11 272	5 2 92 5 27	4.14	2 71	2	2.07	5.23	-			 2	4.71	2.73	-			4 12	3.03	-		+
1100.20	n	IVIPAA	C C	11.323	J 2.J2 J.21	7.10		4	2.00	J.14	1	1		 4	1.54	2.13	1	1	1 1	7.13	3.21			1

## APPENDIX B—LIST OF DENTAL MEASUREMENTS AND AGE ESTIMATES

# APPENDIX C—DENTAL ARCADE PHOTOGRAPHS
















































## APPENDIX D—CURRICULUM VITAE

Name:	Jessica Rose Lacerte
Birthplace:	Rochester Hills, Michigan, USA
Year:	1995
Post-Secondary Education and Degrees:	Master of Arts in Anthropology, University of Western Ontario— 2017-2019 BSc in Anthropology, Eastern Michigan
	University—2015-2017
	Associate Degree in Liberal Arts, Washtenaw Community College— 2012- 2015
Honors and Awards:	Canada Graduate Scholarship—Master's (CGSM-SSHRC)— Fall 2018-Summer 2019
	Christine Nelson Graduate Award (Department of Anthropology, UWO)— Summer 2018
	Ontario Graduate Scholarship (University of Western Ontario)— Fall 2017-Summer 2018
Related Work Experience:	Teaching Assistant

## **Conference Presentations:**

Lacerte, J., Barrett, C. *A Probable Case of Ankylosing Spondylitis among the Peruvian Chiribaya*. Canadian Association of Physical Anthropologists Annual Meeting: Poster presentation. London, Ontario—Oct. 31<sup>st</sup>-Nov. 1<sup>st</sup>, 2018.

Western University

2017-2018

Lacerte, J., Slank, C. *Stature and Sexual Dimorphism as Indicators of Non-Specific Stress among the Peruvian Chiribaya*. American Physical Anthropology Association, Annual Meeting: Poster presentation. Austin, Texas—April 11-14, 2018.

Lacerte, J., Barrett, C., Moore, M. Scanning Electron Microscopy Detection of Linear Enamel Hypoplastic Defects Among a Late Archaic Sample from Ohio. BARFAA conference: Poster presentation. Loyola University, Chicago, Illinois—October 21-22, 2016.

Lacerte, J., Barrett, C. *Estimation of Stature from the Long Bones of the Chiribaya Baja of Southern Peru*. 9<sup>th</sup> World Congress on Mummy Studies: Poster presentation, Lima, Peru—August 10-13, 2016.

Lacerte, J. *Examination of Long Bones of the* Chiribaya *Baja of Southern Peru*. Wayne State University Transformations Graduate Conference: Presenter, Oral presentation. Wayne State University, Detroit, Michigan—March 5, 2016.