Childhood is Biocultural: Refining Our Approaches to the Study of Children in Biological Anthropology

by

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Abstract

Understanding the ontogenetic effects of biocultural factors present in an individual's environment during childhood is essential to the accurate interpretation of lived experiences in past populations. This period of life is critically important for physical and social development, and is characterized by heightened sensitivity to environmental perturbations.

This research explores how skeletal and dental systems respond to biocultural factors throughout early life based on their varying developmental timelines, and tests several longstanding assumptions regarding early development. This is accomplished by investigating recognized skeletal and dental indicators of growth, including long bone length, vertebral neural canal dimensions, and developing tooth length.

This research was conducted using the skeletal remains of approximately 80 children from the Certosa Collection, a documented osteological assemblage of known age and sex from the municipal cemetery in Bologna, Italy. These individuals were known to be from the city's poorest socioeconomic class based on associated records. The findings of this research are interpreted through the biocultural lens, using historical and archival sources to contextualize the physical evidence of the growth environment.

The results demonstrate that first, contrary to existing assumptions, the linear growth of more distally situated bones was not found to have the greatest sensitivity to environmental perturbations, with the proximal bone of the lower limb being the most stunted in this sample. Second, neural canal diameters of the cervical region were found

to have similar or greater levels of diminished growth relative to the lumbar region, which does not align with current methodological practices that exclude cervical data from analyses. Third, development of the dentition is adversely affected by biocultural factors related to an individual's socioeconomic status, with dental development exhibiting increasing variation and delay in tooth length for age.

The findings of this research demonstrate that the physical growth and development of the Certosa children was adversely affected by their poor socioeconomic circumstances. The results from the three scientific articles indicate that existing assumptions in bioarchaeological research require greater exploration and demonstrate the importance of critically evaluating common methodological practices. This research furthers current knowledge of skeletal and dental stress responses to biocultural factors present during childhood, increasing our ability to accurately interpret the lived experiences of children from their physical remains.

Preface

The research project, of which this thesis is a part, received research ethics approval from the University of Alberta Research Ethics Board, Project Name “Biological expressions of childhood social realities in the past from the study of juvenile skeletal remains”, No. Pro00067473.

This thesis is an original work by Jennifer Nelson. Some of the research conducted for this thesis forms part of an international research collaboration, led by Dr. Hugo Cardoso at Simon Fraser University, with Dr. Lesley Harrington being the lead collaborator at the University of Alberta. I declare that I am the main contributor to the three articles that make up this thesis. Chapter four of this thesis is a co-authored paper in the *American Journal of Human Biology*, “Does age estimated from teeth forming in different early life periods show differential discrepancy with known age?,”. Chapter two (Exploring the Laws of Developmental Direction Using a Documented Skeletal Collection) and chapter three (Do the Regions of the Spinal Column Record Stress Differently? An Analysis of Diminished Growth in the Vertebral Neural Canal) are co-authored papers, prepared for submission to the *American Journal of Biological Anthropology*. I am the first author for all three papers, and was responsible for the data collection, preparation, and analysis, with assistance from my co-authors regarding study design and methodology. I wrote the first drafts, prepared tables and figures, and collaborated with the co-authors on manuscript edits and subsequent revisions. Dr. Lesley Harrington was the supervisory author and was involved with concept formation and manuscript composition.

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To anyone I may have missed, please know that it is not for a lack of appreciation but rather a lack of cognitive functioning at this stage of the thesis. Thank you to everyone.

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1. Introduction

Although they make up a large portion of any society, the experiences and contributions of children to their communities were largely overlooked by anthropologists until the late 20th century. Starting around the 1990s, children, along with other previously marginalized groups, began to gain necessary attention (Baxter, 2008; Kamp, 2001; Lillehammer, 1989; Mays et al., 2017; Sofaer Derevenski, 1996). Over the last several decades, anthropologists have focused on increasing our knowledge of childhood and the formative experiences that occur during this phase of life. In particular, their research has centered on studying the physical remains of children from a variety of contexts, recognizing the unique insights this avenue of analysis offers into the lives of individual children, as well as the societies in which they lived.

Childhood is a critical period of the human life course, when an individual undergoes extensive development on both a physical and social level. Biologically, it has been defined as the stage of growth and development extending from weaning through to eruption of the first permanent molar and incisor, during which time an individual will experience consistent skeletal growth, master bipedal locomotion, and achieve final brain volume (Bogin, 2020). Culturally, defining childhood and who is considered a child is not as straightforward. The meaning of these terms is incredibly variable, fluctuating not only between cultures, but also within them over time (Halcrow and Tayles, 2008). For the purposes of this thesis, the term child is used as a broad category for individuals who have not yet reached the age of adulthood and who are still undergoing physical development. This includes infants (0-3 years), children (3-7 years), juveniles (7-12 years), and adolescents (12-20), as defined by Bogin (2020).

It is well established that the growth processes occurring during these early life stages are highly plastic in nature, with both their timing and tempo susceptible to the effects of biocultural factors present in their growth environment, including nutrition, disease, and

generalized stress (Ubelaker, 1987; Goodman and Rose, 1990; Mummert, Schoen, and Lampl, 2018). This susceptibility is heightened in the first stages of life, where the rapid growth and maturation of their developing tissues make them particularly sensitive to perturbations in growth conditions (Buikstra and Ubelaker, 1994; Inglis and Halcrow, 2018). As a result, the bones and teeth of children from past populations provide bioarchaeologists with a physical record of their lived experiences, where measures of growth disruptions or delays serve as a biomarker of early life stress (Lampl and Schoen, 2017; Ubelaker, 1987). These analyses and resulting interpretations extend beyond the life of an individual child, providing insights into the social and environmental conditions of the community surrounding them.

Despite the increase in research devoted to children, there is still much that is not fully understood regarding how their developing dental and skeletal systems respond to variations in their growth environment. Due in part to the subject's relatively recent beginnings, our knowledge of childhood development is also limited by the skeletal material that is available for study. Much of the existing research on children was conducted using archaeological material, where age is determined through estimation methods involving assessments of skeletal and dental development, as chronological age is unknown. This is problematic, as the goal of such research is often to show how skeletal growth and dental development can be adversely affected by biocultural factors, which brings into question the accuracy of the associated estimations of age and resulting interpretations.

Presently, the bioarchaeological study of children has yet to reach levels of understanding and refinement of methodological approaches that are present in our research of the adult members of society. Namely, there are several longstanding assumptions regarding early development which remain to be thoroughly evaluated. These include the assumption that more distally situated bones have a greater sensitivity to negative growth conditions relative to their proximal counterparts, that the neural canal diameters of the lumbar vertebrae have the greatest potential for growth disruption, and that age

estimations based on dental development are not notably impacted by biocultural factors. As these perceptions influence decisions on methodological approaches in research and impact subsequent interpretations of data, it is important that anthropologists critically assess their validity in a range of archaeological contexts.

The purpose of this thesis is to explore these assumptions using the Certosa Collection, a skeletal assemblage of children from low socioeconomic status who lived and died in Bologna, Italy at the turn of the 20th century. The identities of these children, including their sex and chronological age, are known from historical records, such as birth, death, and baptismal certificates (Belcastro et al., 2017). The documented nature of this sample allows for consideration of both social and biological factors when studying the development of these children, in addition to permitting more precise analyses of biomarkers pertaining to size for age. It is intended that the findings of this research will further our abilities to interpret the lived experiences and environmental conditions of children based on the study of their physical remains, while enabling bioarchaeologists to make more informed decisions regarding their methodological approaches in the study of childhood. This will be accomplished through two main research objectives:

1. To identify evidence of diminished growth across a range of developmental systems in the impoverished children of the Certosa Collection and interpret these findings within the socio-historical context of 19th century Bologna, Italy.
2. To investigate whether observations of altered development in the study sample are in line with expectations based on several assumptions regarding patterns of diminished growth which are commonly seen in bioarchaeological research.

My thesis will achieve these objectives by using a biocultural approach to study these children. This interpretative framework recognizes the interplay that exists between human sociocultural forces and our physical beings, where actions and processes occurring in our societies alter our biological state, which in turn impacts our social environment (Goodman and Leatherman, 1998; 2022). By applying the biocultural approach to analyses of children, anthropologists endeavour to interpret physical

evidence of biological processes within the sociocultural contexts in which they occurred, thereby attempting to identify and understand the causal relationships which might be present. My research accomplishes this by examining historical accounts and archival records which describe the social conditions that existed in Bologna at the turn of the 20th century, particularly those which would have impacted the city's impoverished families. This will provide the contextual information necessary to understand the environmental factors impacting the dental and skeletal development of the children in this population.

The research objectives are explored and accomplished in the three scientific articles included in this dissertation. The articles investigate recognized skeletal and dental indicators of growth, including long bone length, vertebral neural canal dimensions, and developing tooth length. The known identities of the Certosa children permit comparative analyses of their anthropometric data with established standards of typical growth and development. Through these comparisons, discrepancies between expected growth and attained growth are identified. Patterns of disrupted growth are examined in these individuals to understand childhood experiences and the impact that biocultural factors related to their poor socioeconomic conditions had on their growth and development. Furthermore, these patterns are then investigated to assess if they follow existing assumptions regarding early development, as outlined below.

The first article (Chapter 2) analyzes the potential effect that developmental order and timing may have on the sensitivity of long bone development to alterations in growth conditions. Existing theoretical frameworks (Kingsbury, 1924; Scammon, 1923) indicate that those skeletal elements located farthest from the head and trunk undergo growth later and at a more rapid rate of development, thereby resulting in them being more sensitive to environmental perturbations. Although this developmental phenomenon is widely accepted amongst the bioarchaeological community and impacts decisions regarding methodological approaches to the study of juvenile development, it is an area of research which remains to be thoroughly evaluated. The second article (Chapter 3) comparatively evaluates variability in the growth of the vertebral neural canal (VNC)

between the regions of the spine. While reduced dimensions of the VNC are frequently interpreted as an indicator that an individual experienced non-specific stress during the first years of their life (Clark et al. 1986), these analyses focus almost exclusively on measurements from the lumbar vertebrae. This can be attributed to the prevailing belief that this region of the spine has the greatest potential to record growth disruptions due to its lengthy window of postnatal development. Despite the pervasiveness of this assumption, there are no studies which comparatively evaluate evidence of growth disruptions of all three vertebral regions. The third and final article (Chapter 4), 'Does age estimated from teeth forming in different early life periods show differential discrepancy with known age?' is published in the *American Journal of Human Biology* (Nelson et al., 2021). This study explores the differential sensitivity of developing teeth to biocultural factors present in the growth environment. While frequently cited as being less sensitive to environmental perturbations than skeletal elements (Cardoso, 2007a), the extent to which dental development is impacted by such factors has not been fully established.

The remainder of this introduction provides necessary background information for this research, including skeletal and dental development, the anthropological study of these processes, and contextual information pertaining to the study sample. The fifth and final chapter of this dissertation presents a summary of the research findings, discussing the outcomes of each article and synthesizing how these studies contribute to our understanding of children and their biocultural experiences.

By examining commonly employed biomarkers in the context of a known age and sex sample, this dissertation investigates the complex process of early development and furthers our knowledge of how the developing body responds to conditions within the growth environment. Through this research, our understanding of how these developmental systems are impacted by biocultural factors experienced during this critical life period is advanced. These studies will inform our methodological choices in bioanthropological studies of children, in addition to improving our ability to interpret the

experiences of these individuals from their physical remains and add to our knowledge of what childhood was like in the past.

1.1. Childhood Growth and Development

The rapid growth and high levels of cellular activity experienced in developing bones and teeth make them particularly susceptible to environmental conditions, and as such, their study can provide information regarding a child's development, health, nutrition, and surroundings (Buikstra and Ubelaker, 1994; Inglis and Halcrow, 2018). Exposure to poor growth conditions and early life stress may impact the timing, rate, and quality of growth in dental and skeletal tissues (Cardoso, 2007a; Lampl, 2022; Šešelj, 2013). The human skeleton and dentition begin their development within weeks of conception and do not reach their final adult form until the third decade of life (Bhattacharya and Stubblefield, 2016; Cunningham et al., 2017). This broad developmental window means the study of an individual's bones and teeth is not only useful for our understanding of their life in the decades after birth, but can also offer insight into in utero development and maternal health (Larsen, 2015). In order to assess childhood growth across social and developmental stages, biological anthropologists rely on a variety of skeletal and dental elements, as their formation plays out over varied tempos (Ubelaker, 1987).

The development of the human body occurs through complex processes, where our genes direct the timing and rate of growth in various bodily systems (Lampl and Schoen, 2017). This genetic control is more evident in the fetal and early infant developmental stages, gradually lessening with increasing age (Eveleth and Tanner, 1990). In addition to genetics, the developmental process is also quite susceptible to environmental variables such as nutrition, illness, and socioeconomic factors, both in utero and in postnatal life (Mummert, Schoen, and Lampl, 2018). For proper growth potential to be achieved, homeostasis needs to be maintained across bodily systems throughout development (Eveleth and Tanner 1990). Through clinical and archaeological research, the timelines of skeletal and dental growth have been established, enabling biological anthropologists to not only estimate the age of immature individuals based on the developmental stage of

their bones and dentition, but also detect growth stunting, estimate stature, and identify evidence of illness (Lampl and Schoen, 2017; Ubelaker, 1987).

Although our knowledge of ontogenetic processes has increased markedly in recent decades (Inzaghi et al., 2022; Long and Ornitz, 2013; Millward, 2017), there are still areas in which our understanding is incomplete. In particular, there remain uncertainties in regard to how the various factors related to an individual's biocultural circumstances impact the growth of their bones and teeth. Existing research on this subject (Cardoso, 2007a; Clark et al., 1985; Lampl and Schoen, 2017) has highlighted the complex and multi-faceted nature of developmental processes, with the final outcome of growing tissues resulting from the interplay of genetic and environmental factors (Lampl, 2022; Leatherman and Goodman, 2022). This research has also identified potential patterns (Cardoso, 2007a; Clark et al., 1985; Conceição and Cardoso, 2011; Šešelj, 2013; Smith and Buschang, 2004; Tanner, 1978) in how developing skeletal and dental tissues respond to these various stimuli. From these observations, several theoretical assumptions have formed regarding the ways in which various developmental processes, including long bone growth (DeWitte, 2018; Kingsbury, 1924; Lampl, Kuzawa, and Jeanty, 2003; Scammon, 1923), vertebral development (Clark et al., 1985; Watts, 2011), and tooth formation (Cardoso, 2007a), occur when an individual experiences conditions of stress. These include the assumption that there is a greater sensitivity to growth perturbations in distal skeletal elements, that the lumbar vertebrae are more likely to record evidence of disrupted growth than the cervical and thoracic vertebrae, and that age estimated from the dentition is not altered by exposure to biocultural factors. These assumptions have the potential to alter the outcome of bioarchaeological research into the experiences of past peoples by influencing key methodological choices, particularly the selection of skeletal elements for analysis, as well as interpretations which are based on estimated age. Because of this, it is important that the validity of these assumptions is critically evaluated in a range of populations and biocultural contexts, such as the one explored through this doctoral project. The research background and premises of these assumptions will be outlined in the following three subsections.

1.1.1. Development of the Long Bones

To interpret evidence of exposure to stress or poor growth conditions, it is first necessary to understand the mechanisms and timelines of skeletal development. The formation of the human skeleton begins during the embryonic phase, which encompasses the first 8 weeks in utero (Cunningham et al., 2017; Jin, Sim, and Kim, 2016). Towards the end of this phase, a hyaline cartilaginous precursor of the long bones is formed by cartilage depositing cells known as chondroblasts (Betts et al., 2013). Via a process known as endochondral ossification, this hyaline cartilage is gradually replaced by bone tissue through the actions of bone-forming cells or osteoblasts. As these osteoblasts begin to deposit osteoid onto this cartilaginous model, the original cartilage cells die and disintegrate, leaving behind the newly formed bone (Ortega, Behonick, and Werb, 2014).

To maintain their functionality and basic shape throughout the growth process, bones which are formed through endochondral ossification must increase in both diameter and length. The shaft, or diaphysis, increases in diameter by appositional growth, where osteoblasts deposit new bone tissue on the outer surface of the shaft, while bone is being resorbed from the inner cavity by osteoclasts (Betts et al., 2013). Elongation of a bone happens at its metaphyseal growth plates, which are located at the proximal and distal ends of the bone, between the diaphysis and epiphyses (Allen and Burr, 2019). These plates are made of hyaline cartilage, which produces additional cartilage matrix through the life cycle activities of chondrocytes (Betts et al., 2013), located in the area of the growth plate that is closest to the bone's articular surface (known as the "Resting Zone"). At the beginning of their cell cycle, the purpose of chondrocytes is to act as matrix secretory cells. In response to biological signals, some of these chondrocytes will then shift their function to self-replication ("Proliferative Zone"), after which they will exit the cell cycle as a unit, aligning themselves along the bone's long axis (Salhotra et al., 2020). This is referred to as the "Pre-Hypertrophic Zone", where these units of chondrocytes synthesize the protein within the matrix. Following this stage, chemical signals will trigger hypertrophy of these cells ("Hypertrophic Zone"), causing the chondrocyte units to

increase in their size by 5- to 20- fold (Lampl, 2018). These enlarged cells are found at the edge of the growth plate nearest to the diaphysis of the bone. From here, bone apposition is achieved through the actions of osteoblasts, which deposit organic matrix along the existing structure of hypertrophic chondrocyte cells (Salhotra et al., 2020). This organic matrix will then be mineralized and remodeled by bone-resorbing cells known as osteoclasts in the "Ossification Zone" (Lampl, 2018). This process will repeat itself throughout an individual's developmental period, elongating the bone with the completion of each cell cycle. At a developmentally determined point, the growth plate will cease its production of chondrocytes, and subsequently of cartilage, after which the epiphyses will fuse to the diaphysis and the bone will be unable to grow in length (Lampl and Schoen, 2017).

While it is established that stress has a negative effect on skeletal development, the precise mechanism of this relationship is not yet fully understood, however ongoing research has produced basic principles upon which educated hypotheses can be made (Lampl and Schoen, 2017; Mousikou, Kyriajou, and Skordi, 2023; Sinclair and Dangerfield, 1998; Tsukasaki and Takayanagi, 2019). Whether the stress being experienced is of a physical or a psychological origin, the outcome is the same; a reduction in growth resulting in skeletal dimensions below what would be considered normal at that age (Bogin, 1999). This may be due in part to the engagement of the higher brain centres via the hypothalamus-pituitary-adrenal axis (HPAA), which can be activated by any stressor, psychological or physical (Mousikou, Kyriajou, and Skordi, 2023). In response, the amygdala and the limbic cortex are affected, which transfer nerve impulses to the hypothalamus, which in turn sends out neuroendocrine messages in the form of stress hormones, otherwise known as corticosteroids (Ponzi et al., 2020). These hormones signal that the body's energy and resources should be redirected from growth processes to systems critical for survival, causing the pituitary gland to halt the secretion of growth hormones (Bogin, 1999; Mousikou, Kyriajou, and Skordi, 2023; Ponzi et al., 2020). When this occurs, the hypertrophy of chondrocytes at the growth plates will be reduced or ceased, resulting in diminished elongation of the bone (Abe et al., 2019).

If the stress continues, it is possible that the individual will effectively miss out on that growth event or episode. The likelihood of this occurring is dependent on when during a skeletal element's developmental timeline the stressor is experienced. Skeletal elements which complete their growth early in life and at more rapid rates are less likely to recover from growth insults, as are skeletal elements whose growth is disrupted towards the end of their developmental window (Cameron, 2012; McPherson, 2021). Research into bone growth has shown that when stress ceases sufficiently early within an element's developmental window, there is the capacity for the growth event to resume through a reversal of the chondrocytes to the previous stage in their life cycle (Lampl and Schoen, 2017). In these instances, the bones have the potential to grow at an accelerated rate and catch up to their growing potential (Tanner, 1981). Individuals who survive acute stress incidents almost always resume their original pattern of growth, attaining approximately the same height predicted based on their initial growth trajectory (Cameron, 2012). This is known as "canalisation", which is believed to be caused by genetically controlled patterns of growth, where the potential for adult stature is a target that our bodies will naturally try to reach (Hermanussen, Largo, and Molinari, 2001; Wit and Boersma, 2002). The amount of deviation from this target is dependent on the duration, severity, and timing of the stress insults experienced by the individual (Cameron, 2012). What is not clear however, is if the bone that is produced during these "catch-up" events is of an equal quality to bone formed in the absence of stress (Lampl and Schoen, 2017).

Beyond their potential to activate the HPA regulatory system, some physiological stressors may alter bone development in other ways. In particular, researchers have endeavoured to understand the impact that specific diseases and associated physical symptoms have on skeletal growth processes. These stressors have been shown to impact bone growth in several ways, with both clinical and population-based studies demonstrating the deleterious impact that illness has on growth (DeBoer et al., 2017; Stephensen, 1999). Research has shown that inflammation of the gastrointestinal system, also referred to as gastroenteritis, triggers the suppression of osteoblastic activity, thereby slowing or reducing the formation of bone (Tsukasaki and Takayanagi, 2019).

Similar findings have been observed in instances of viral infections, such as pneumonia, where an increase in cytokine levels results in activation of the immune response system, causing a disruption to signaling pathways and reduction in bone formation (Millward, 2017; Tsukasaki and Takayanagi, 2019). In individuals with prolonged inflammatory responses, inhibition of chondrocyte proliferation and thinning of the epiphyseal plates occurs, resulting in fewer chondrocytes available for cartilage production and a significant reduction in elongation at the growth plate (Irwin et al., 2016; Sinclair and Dangerfield, 1998). This has been linked to the inhibition of bone promoting growth hormones, such as growth hormone (GH) and insulin-like growth factor 1 (IGF-1) (Cuestas et al., 2023; Wong et al., 2016). These low levels of GH and IGF-1 are shown to be further exacerbated by a resistance to these hormones in the growth plate, believed to be the result of pro-inflammatory cytokines (Cirillo et al., 2017).

Research has also demonstrated a link between malnutrition, disease, and delayed bone formation. As a result of compromised immune systems, poorly nourished children are more susceptible to and more severely affected by illness (Eveleth and Tanner, 1990; Lampl and Schoen, 2017). Additionally, low appetite is associated with disease, further exacerbating this cycle of influence (Millward, 2017; Stephensen, 1999). Extensive research involving human and animal models has demonstrated the necessity for adequate nutrition for proper bone development (Adriani and Wirjatmadi, 2014), where cellular activity at the growth plate and rates of bone elongation are impacted by an individual's dietary intake (Millward, 2017). Research has found that even a 20% reduction in caloric intake below daily recommended values can result in diminished linear skeletal growth (De Luca, 2006; Betts and Magrath, 1974). This has been attributed to reduced numbers of chondrocytes and observable thinning of the growth plate (Farnum et al., 2003). Additionally, diets which are low in fat and nutrients have been shown to result in inadequate levels of IGF-1, the outcome of which is improperly functioning growth plates and a reduction in bone growth (Racine and Serrat, 2020; Rossi et al., 2001). Similar findings have also been observed in studies exploring the importance of protein for skeletal growth, where diets which were protein deficient, such as those

reliant on grains or starches with little animal-food sources, saw an inhibition of linear growth due to insufficient IGF-1 levels and poor growth plate activity (Tirapegui et al., 2012; Yahya and Millward, 1994). As with growth delays resulting from activation of the HPA axis, if an individual is still within the developmental window when the illness or malnourishment cease, the bones will undergo accelerated growth to catch up to their pre-stress genetic potential, as per the process of canalisation previously outlined (Cameron, 2002).

In many studies of human growth (Bogin et al., 2002; Cardoso and Magalhaes, 2011; Cardoso et al., 2021; Holiday, 1999; Gowland, 2015; Higgins and Ruff, 2011; Jantz and Jantz, 1999; Meadows and Jantz, 1995; Pomeroy et al., 2012; Rios et al., 2020; Smith and Buschang, 2004; Tanner, 1978), a trend has been noted in how limb growth responds to stressful conditions and fluctuations in the biocultural environment. Differential variation has been observed in samples of both living and deceased individuals, where the distal segment of the upper and lower limb shows greater variability in attained length relative to that of the proximal segment. This is particularly true of the tibia, which has been found to exhibit the most variability of all long bones in many studies (Auerbach and Sylvester, 2011; Bogin et al., 2002; Meadows and Jantz, 1995; Schweich, 2000; Ulijaszek, 1998). Based on these findings, the tibia is often considered to have the greatest sensitivity to biocultural factors relative to the other long bones (Cardoso and Magalhaes, 2011; Gowland, 2015; Jantz and Jantz, 1999). In studies of modern samples, this increased sensitivity has been observed in both segments of the lower limb, with several articles finding greater variability in leg length relative to that observed in arm length (Bogin et al., 2002; Bogin and Varela-Silva, 2010; Buschang, 1982; Frisancho, 2007; Li, Dangour, and Power, 2007; Jantz and Jantz, 1999; Smith and Buschang, 2004; Tanner et al., 1982).

Although the tibia is frequently referenced as being the most sensitive bone to growth disruptions, and has often been demonstrated as such in existing studies, explanations for this differential sensitivity are not commonly seen in the literature. Some which have been proposed include a reduction in blood flow to distally located elements (Pomeroy et

al., 2012; Holliday and Ruff, 2001; Bogin and Varela-Silva, 2010), environmental adaptations for thermoregulation (Holliday and Ruff, 2001; Temple et al., 2008; Pomeroy et al., 2012), and the cephalo-caudal principle of growth (Bogin and Varela-Silva, 2010; Schweich, 2005; Jantz and Owsley, 1984). While all three have merit and may play a role in the differential sensitivity observed in growing bones, it is the latter hypothesis which appears most frequently in research evaluating length attainment as an indicator of non-specific stress. This explanation ascertains that the differing developmental timelines and growth tempos of skeletal elements is responsible for their variable sensitivity to growth perturbations (Seeman, 1997; Smith and Buschang, 2005; Bogin and Varela-Silva, 2010). The human body grows in a cephalo-caudal sequence, with development of tissues located superiorly occurring prior to that of tissues at the inferior or "tail" end (Kingsbury, 1924). A similar sequence of growth occurs in the appendicular skeleton, which follows a proximodistal order of development (Scammon, 1923). Commonly referred to as the *laws of developmental direction* (Kingsbury, 1924; Scammon, 1923), the outcome of these patterns in growth is that skeletal elements located farthest from the trunk have completed less of their development at birth. Consequently, these elements experience greater postnatal growth at a more rapid rate than elements situated nearer to the head and trunk (Kingsbury, 1924). This faster rate of growth results in these bones having greater sensitivity to fluctuations in growth conditions after birth (Cardoso and Magalhaes, 2011; Bogin, 1999; Eveleth and Tanner, 1990; Smith and Buschang, 2004).

This theoretical framework has shaped approaches to the study of growth in past and present populations. Notably, the belief that the tibia has the greatest sensitivity has led to several studies using only tibial measurements for their research (DeWitte, 2018; Lampl, Kuzawa, and Jeanty, 2003; Mensforth, 1985; Newman, Gowland, and Caffell, 2019; Pomeroy et al., 2014), rather than collecting data from all long bones. This is based on the perception that the tibia has the greatest potential for recording evidence of disrupted growth. While there have been studies whose results align with the pattern of greater variability in tibial length (Cardoso and Magalhaes, 2011; Gowland, 2015; Jantz and Jantz, 1999), there are also several studies with findings that do not support this. Research

completed in 2019 (Gooderham et al.) found that Portuguese children from the medieval period had femora which exhibited more variability and diminished growth than that of their tibiae. Similar findings were observed in a sample of impoverished children from 20th century Portugal (Cardoso, 2005). A comparative analysis of skeletal growth in temporally distinct foraging groups from Siberia during the Neolithic found that while the femora displayed significant levels of growth stunting linked to systemic stress, the tibiae were not found to be significantly stunted in length (Temple et al., 2014). Research evaluating the impact of structural violence on growth (Cardoso et al., 2018) in populations of enslaved African children in Portugal and impoverished black communities from 1920s America, found greater femoral stunting relative to the tibia in two of the three samples analyzed. It is apparent from the findings of these and other studies (Holliday and Ruff, 2001; Johnston et al., 1962; Pinhasi et al., 2006) that approaches to bioarchaeological research based on the *laws of developmental direction* warrant additional exploration. Specifically, by evaluating osteological evidence of disrupted growth using this theoretical framework across a range of biocultural contexts, including the one examined in this doctoral research, we stand to better comprehend the impact that ontogenetic processes and environmental conditions have on skeletal development. Through this additional understanding, biological anthropologists will be better equipped to make appropriate methodological decisions when designing their studies, thereby maximizing the information gleaned from research involving the physical remains of children and more effectively interpreting their experiences.

1.1.2. Development of the Vertebral Neural Canal

Neuro-osseous tissues are among the first to form in utero (Cunningham et al., 2017). This includes the vertebral elements of the spine, which appear as chondrification centres at approximately six weeks after conception (Louryan, Vanmuylder, and Bruneau, 2011). Each vertebrae begins as three separate centres, which go on to form the vertebral body or centrum, and the two halves of the neural arches (Cunningham et al., 2017). By the end of the second month in utero, these chondrification centres have expanded and

fused to form a single cartilaginous vertebra around the spinal cord, creating the neural canal (Louryan, Vanmuylder, and Bruneau, 2011; Cunningham et al., 2017). Through endochondral ossification, the cartilaginous vertebra model is converted into bone by osteoblasts through the secretion of osteoid, forming three primary ossification centres, one for the centrum and two for the neural arches (Cunningham et al., 2017; Williams; Alkhatib, and Serra, 2019).

Of the three vertebral regions, it is the cervical which begins ossifying first, with the cervical neural arches visible midway through the second month in utero (Cunningham et al., 2017). Ossification of the neural arches proceeds down the spine in a caudal direction, with the thoracic neural arches showing ossification occurring during the third fetal month (Bagnall et al., 1977). It is also in the third month in utero that the centra begin to ossify, commencing in the low thoracic region around the ninth fetal week (Louryan, Vanmuylder, and Bruneau, 2011). Centra ossification progresses outwardly from here in the cranial and caudal directions, reaching the centrum of the fifth lumbar vertebra around twelve weeks in utero and the cervical centra in the fourth fetal month (Cunningham et al., 2017). It is also during the fourth fetal month that the lumbar neural arches undergo ossification (Cunningham et al., 2017).

Through the process of endochondral ossification, the neural arches and centrum increase in size via activity of chondroblasts and osteoblasts at the three vertebral growth plates (Cunningham et al. 2017; Maat et al., 1996). Located at the neurocentral junction and posterior synchondrosis, these cartilaginous growth plates expand through the secretion of cartilage matrix by chondrocytes, which then undergo proliferation and hypertrophy along the edge of the developing bone (Labrom, 2007; Salhotra et al., 2020). Osteoblasts then deposit organic matrix into the structure made up of hypertrophic chondrocytes, which is mineralized and remodeled through osteoclastic activity (Lampl, 2018). Through the endochondral growth of the three vertebral elements, the transverse (TR) and antero-posterior (AP) diameters of the developing neural canal expand in size (Cunningham et al., 2017). This growth will continue until fusion occurs at all junctions.

Growth occurs first between the neural arches, which fuse posteriorly during the first year after birth (Dimeglio, 1992). Neural arches in the lower thoracic and upper lumbar vertebrae are the first to complete posterior fusion during the latter half of the first postnatal year, with the process continuing in successive order towards the cranial and caudal directions (Cunningham et al., 2017). This process is complete in the thoracic region by the end of the second year of postnatal life (Cunningham et al., 2017). Posterior fusion of all cervical arches occurs in the second and third years after birth, while the lower lumbar vertebrae have been documented to fuse posteriorly as late as the end of year five (Cunningham et al., 2017; Ursu et al., 1996). Once posterior fusion is completed, the TR diameter of the neural canal is largely established, with research indicating that upwards of 95% of adult size for this dimension is attained by this point, commonly by the age of six years (Dimeglio, 1992).

The neural canal will continue to increase in size in the AP dimension until fusion is completed between the neural arches and vertebral body at the neurocentral junction. This occurs first in the lumbar vertebrae, beginning during the second year of life with complete fusion observed in this region by age four (Cunningham et al., 2017). The cervical region is the next to undergo neurocentral fusion, typically between three and four years of age (Cunningham et al., 2017). The thoracic vertebrae are the last to complete fusion of the neurocentral junction, generally between ages four and five (Baker, 2005). After six years of age, all neurocentral junctions will have completed fusion, with approximately 95% of adult size attained by this age (Dimeglio, 1992).

Once fusion occurs at all three growth plates, the dimensions of the neural canal are largely “locked in”, with minimal increases in size occurring after the age of six years (Dimeglio, 1992; Newman and Gowland, 2015). After this point, the neural canal changes primarily in shape rather than size, undergoing bone remodeling via intramembranous ossification (Reichmann and Lewin, 1971). During this process, the dimensions of the neural canal may be altered through resorption of the vertebral body’s inner surface (Reichmann and Lewin, 1971), which might contribute to an increase in diameter size

with age. Research has demonstrated that these post-fusion changes occur to different extents in the AP and TR diameters (Dimeglio, 1992; Newman and Gowland, 2015). The AP dimension has not been found to increase in size after fusion has completed between the neural arches and centrum (Dimeglio, 1992). In comparison, small increases in size of the TR dimension have been shown to occur, with several studies concluding that final dimensions in this diameter are not reached until between 15 and 17 years of age (Hinck, 1966; Newman and Gowland, 2015; Watts, 2013).

The dimensions of the vertebral neural canal (VNC) are used as a biomarker of an individual's health in early life, with small diameters serving as evidence of stress having occurred during the vertebral developmental windows. This stems from foundational research conducted on young pigs by Platt and Stewart (1962), which demonstrated an association between VNC development and undernutrition, with pigs who received a protein-calorie deficient diet exhibiting a 17-21% deficit in VNC size relative to pigs who were fed a normal diet. The application of these findings was first explored in human populations by Clark et al. (1986), who examined the impact of shifting subsistence patterns and health on the VNC dimensions of a prehistoric population. Their study demonstrated a correlation between lifespan and diameters of the VNC, where individuals with reduced VNC dimensions were found to have died significantly earlier than individuals with larger neural canals (Clark et al. 1986). Research also indicates that the relatively short and rapid developmental timeline of neuro-osseous tissues, including vertebral elements, make them particularly sensitive to growth disruptions due to biocultural factors, including malnutrition, disease, and poor environmental conditions (Brooke et al., 1984; Clark et al., 1986; Papp et al., 1994; Platt and Stewart, 1962). Since the work of Clark and colleagues, the use of VNC diameters as an indicator of early life stress has been seen in many bioarchaeological studies (Amoroso and Garcia, 2018; Clark et al., 1988; Watts, 2011, 2013; Newman and Gowland, 2015), with small dimensions found to be associated with poor growth and increased risk of mortality.

Although small VNC diameters are frequently noted in both clinical studies of living patients (Amonoo-Kuofi, 1982, 1985; Muthuuri, 2021; Oguz et al., 2002; Porter et al., 1987b; Ranjan Das et al., 2018; Van Roy et al., 2001; Verbiest, 1980) and research involving the physical remains of past populations (Clark et al., 1986, 1987; Corron, Wolfe, and Stull, 2021, 2023; Newman and Gowland, 2015; Papp, Porter, and Aspden, 1994; Porter and Pavitt, 1987a; Rewekant, 2001; Watts, 2011, 2013a, 2013b, 2015), it was not possible to locate any publications which discuss the biological mechanisms of how stress results in growth disruptions specific to the VNC. It has been suggested that as the vertebral elements complete their growth through the same osteological processes as long bones (Bick and Copel, 1950; Newman and Gowland, 2015), stress experienced during early life leads to disrupted growth of vertebral elements through similar mechanisms as those identified in endochondral ossification of the appendicular skeleton. As was outlined in the previous section, when exposed to non-specific stress, the body prioritizes those functions and systems necessary for survival over non-vital processes, such as skeletal growth (Ponzi et al., 2020). This is achieved through activation of the HPAA, resulting in the release of corticosteroids by the hypothalamus, which in turn leads to a cessation of growth hormones by the pituitary gland (Bogin, 1999; Mousikou, Kyriajou, and Skordi, 2023). The result of this is a slowing or cessation of chondrocyte and osteoblast activity at the growth plates, which in turn leads to a reduction in growth (Lampl and Schoen, 2017). Similar outcomes have been observed in instances of malnutrition and illness, both of which have been shown to have direct impacts on endochondral ossification processes via their role in the release of growth stimulating hormones (Cuestas et al., 2023; Racine and Serrat, 2020; Tirapegui et al., 2012; Tsukasaki and Takayanagi, 2019). In the vertebral elements, this would cause the neural arches and centrum to grow smaller than their potential, resulting in a neural canal with diameters smaller than would be expected for an individual undergoing unimpeded growth.

As with growth of the long bones, it may be possible for normal vertebral growth to resume and neural canal size potential to be achieved if the source of the stress is alleviated. Unfortunately, as with our understanding of stress mechanisms and vertebral

development, there is a lack of information available regarding catch-up growth specific to vertebrae. Research examining catch-up growth of neuro-osseous tissues, which include vertebral elements, indicates that due to their short developmental window, alleviation of stress must occur prior to the age of two for the individual to attain their original pattern of growth (Brandt, 1978; Clark, 1985). This is due to the fact that the potential for catch-up growth is dependent on the timing of growth disruption and how much time is remaining in an element's developmental period at the time of stress cessation (Brandt, 1978; Clark, 1985). As the majority of neural canal development occurs within the first years of life, this greatly limits the potential for catch-up growth to occur in VNC diameters of individuals experiencing disrupted growth during infancy.

Some individuals have suggested that catch-up growth may occur in the transverse diameter, as this dimension has been found to increase in size up until the age of 15-17 years (Hinck et al., 1966; Newman and Gowland, 2015; Watts, 2013b). From a developmental perspective, interpreting these documented increases as "growth" may not be wholly accurate. As outlined previously, once fusion has occurred at the posterior and neurocentral growth plates, changes observed in the neural canal are due to remodeling of the vertebral elements (Reichman and Lewin, 1971). This remodeling results in changes to the shape of the canal with age (Cunningham et al., 2017; White et al., 2011). While the cervical and thoracic canals experience only minor changes, the lumbar neural canals undergo substantial remodeling, changing in shape from ovoid to triangular, with the longest border parallel to the transverse axis (Reichmann and Lewin, 1971; Porter and Pavitt, 1987a; White et al., 2011). This remodeling process has a pronounced effect on the transverse diameter, which increases in size due to resorption of bone from the medial edges of the neural canal (Reichmann and Lewin, 1971). This means that post-fusion increases in size which have been observed in this dimension are not the result of additional growth in the vertebral elements, which brings into question whether they can be viewed as evidence of catch-up growth. Additionally, research design and interpretations regarding an individual's growth environment and biocultural

experiences based on these post-fusion increases in size should be evaluated in light of this.

Although there has been increasing recognition of the insights possible through analyses of VNC diameters in both living and dead populations, research involving neural canal dimensions has been limited in its scope. Many studies (Corron, Wolfe, and Stull, 2021, 2023; Papp, Porter, and Aspden, 1994; Porter and Pavitt, 1987b; Rewekant, 2001; Watts, 2011, 2013a, 2013b, 2015) which use VNC diameters to assess population health analyze measurements from the lumbar region alone, without examining data from the cervical and thoracic vertebrae. This prioritization of lumbar measurements has been attributed to the relatively longer growth period of lumbar vertebrae when compared to those of the cervical and thoracic regions. This lengthy period of postnatal development has been interpreted as the lumbar vertebrae having the greatest potential for recording episodes of diminished growth (Clark et al., 1985; Watts, 2011).

The perception that lumbar vertebrae are more likely to record experiences of non-specific stress has greatly influenced methodological decisions in bioarchaeological research. Notably, only a handful of publications (Amoroso and Garcia, 2018; Newman and Gowland, 2015) could be identified which analyze measurements from all three vertebral regions. To date, there are no studies which comparatively evaluate diminished VNC growth of the cervical, thoracic, and lumbar vertebrae in either dimension. Therefore, the prevailing belief that the lumbar vertebrae are the most useful for detecting growth disruption and poor health has not been critically investigated. As the diameters of the spinal regions undergo their development on differing timelines, this methodological approach may be overlooking vital information pertaining to stress episodes which occurred during the cervical and thoracic developmental windows. If the purpose of bioarchaeological research is to understand the experiences of individuals from the past, it is important that we examine all parts of an individual's life recorded by their remains.

1.1.3. Development of the Dentition

Initial formation of the oral cavity begins around 4 weeks in utero, with dental development beginning shortly thereafter (Cunningham et al., 2017; Hillson, 1996). The dental lamina appears at approximately 6 weeks gestational age, where portions of epithelium begin to thicken and penetrate into its underlying mesenchyme, creating tooth germs (Balic, 2019; Hillson, 2014). Numbering twenty in total, each of these germs will form into a deciduous tooth bud, with the tooth germs for the permanent dentition appearing at roughly the 16th week in utero (Kraus, 1959; Smith, 1991). These buds then pass through developmental stages commonly referred to as the "bud, cap, and bell" stages, during which time key structures begin to form (Baranova et al., 2020). It is during the final "bell stage" that the shape of the future cusps and tooth crown begin to form (Hillson, 1996). It is also at this point that dentine-secreting odontoblasts and enamel-forming ameloblasts appear (Cunningham et al., 2017).

Crown formation begins with the deposition of collagenous predentin by odontoblasts at the tip of each tooth cusp, progressing gradually towards the cervical edge or neck of the tooth (Bartlett, 2013). This predentin form then mineralizes, providing a structure upon which ameloblasts secrete enamel matrix during the secretory stage of tooth development. Composed of protein, water, and calcium phosphate in the form of hydroxyapatite (HA) crystals, this matrix is deposited in layers beginning at the cusp tips; a process which is known as amelogenesis (Bartlett, 2013). Once the cemento-enamel junction (CEJ) has been reached, ameloblasts stop producing enamel matrix and their function switches to crown mineralization (Baranova et al., 2020; Goodman and Rose, 1990).

Beginning at the cusp tips or incisal edge, the ameloblasts spread downward until the entire crown is mineralized (Liversidge and Molleson, 1999). After this process is complete, the ameloblasts expand and die, resulting in an acellular inorganic tissue that is incapable of remodelling (Baranova et al., 2020; Goodman and Rose, 1990). The first of the deciduous teeth, the first and second incisors, complete their crown development

around 2 to 4 months after birth (AlQahtani, Hector, and Liversidge, 2010). These are followed shortly by the third deciduous premolar (also referred to as the first deciduous molar) at approximately 6 months, and the deciduous canine soon after at roughly 8 to 10 months of age. The fourth deciduous premolar (also known as the second deciduous molar) is the last of the deciduous dentition to complete its crown formation, occurring at around the tenth or twelfth postnatal month (AlQahtani, Hector, and Liversidge, 2010).

While the deciduous dentition undergoes the majority of its development in utero, the permanent teeth do not begin developing until after the child is born. By the end of the first year of life, the crowns of the permanent incisors, canines, and first molars have all begun development (Massler and Schour, 1946). The first molars begin their initial crown formation around the time of birth, completing their crowns at approximately 2 to 4 years of age (Gustafson and Koch, 1974). By the 2 to 4 month mark after birth, all anterior teeth, excluding the upper second incisors, have begun crown formation (Liversidge, 2000). The upper second incisors do not begin their crown development until around 10 months of age (AlQahtani, Hector, and Liversidge, 2010). All incisor crowns are complete by approximately 3 to 5 years of age, while the crowns of the canines are not fully formed until around 4 to 7 years (Gustafson and, Koch 1974). At roughly 2 to 4 years after birth, the premolars and second molar begin their crown development, achieving full crown completion at approximately 6 to 8 years of age (Massler and Schour, 1946). Beginning at around 7 to 9 years of age, the third molar is the last of the dentition to undergo crown formation, and does not achieve full crown formation until early adolescence, although the range of development completion is incredibly variable (Massler and Schour, 1946).

Research conducted over the past several decades has demonstrated the impact that various biocultural factors have on dental development. Through the use of both animal and human models, these studies have increased our understanding of how exposure to stressors within an individual's growth environment alters their tooth formation. Developing dental structures show fluctuations in their growth in response to systemic disturbances, resulting in stunted tooth length, delayed alveolar eruption, and

hypoplastic defects of the hard tissues (Cardoso, 2007a; Conceição and Cardoso, 2011; Hillson, 2014; Lewis and Garn, 1960; Sarnat and Schour, 1941; Šešelj, 2013). In comparison to skeletal development, there has not been as much research regarding the direct effects of stress on dental formation. Several signaling pathways and gene proteins involved in the process of tooth morphogenesis, however, have been identified (Chhabra et al., 2014; Jernvall and Thesleff, 2000). Studies indicate that tooth formation is regulated by the wingless-related integration site (Wnt) pathway (Moriguchi, Yamada, Miake, and Nitta, 2011; Suomalainen and Thesleff, 2010; Yang et al., 2018), transforming growth factor (TGF) (Tummers and Thesleff, 2009; Yang et al., 2018), bone morphogenetic protein (Bmp) (O'Connell et al., 2012; Tummers and Thesleff, 2009), fibroblast growth factor (FGF) (Jarvinen et al., 2006; Tummers and Thesleff, 2009), and sonic hedgehog (Shh) (Jarvinen et al., 2006; Tummers and Thesleff, 2009). These pathways regulate the activities of ameloblasts throughout the process of amelogenesis, controlling the production of enamel matrix (Moriguchi et al., 2011; Yang et al., 2018). When an individual is exposed to stress, it may result in the abnormal functioning of these signaling pathways, which in turn has the potential to lead to disruptions in the development of dental tissues. Irregular activation or inhibition of signaling pathways occurring during the secretory stage of crown formation may prevent normal ameloblast activity, resulting in a reduction or cessation in the secretion of enamel matrix, with normal activity only resuming once the stress ceases (Ritzman et al. 2008; Tummers and Thesleff, 2009). When this occurs, tooth crown formation may be impacted and overall tooth length reduced.

Irregularity in signaling pathway functioning can result from exposure to various biocultural factors, including malnutrition, illness, and stressors which are psychological in nature (Ponzi et al., 2020; Ryyanen et al., 2014; Tung et al., 2006; Yuii, Suzuki, and Kurachi, 2007). As was outlined in the previous section on skeletal growth, episodes of stress may alter development of the dentition through activation of the HPA axis (Temple, 2019). This adaptive response system redirects energetic resources, prioritizing bodily systems necessary for survival while slowing or suppressing non-essential processes, such

as tooth formation. This is accomplished through the release of corticosteroids, commonly referred to as cortisol (Ponzi et al., 2020). Research has shown that these stress hormones lead to reduced levels of insulin-like growth factor, which slows ameloblast activity and disrupts the secretion of enamel matrix (Al-douri, Al-Salihi, and Mohammed, 2005; Yamamoto, 2006; Yuii, Suzuki, and Kurachi, 2007). The outcomes of this process have been observed in children from families of lower socioeconomic status, who were found to have thinner and hypomineralized enamel as a result of increased levels of cortisol secretion (Boyce et al., 2010).

In addition to the HPA response system, the role that nutritional intake and environmental conditions play in the functioning of ameloblasts and their production of enamel has been evaluated. Findings from animal and in vitro human studies reveal that adequate levels of calcium are necessary for ameloblast differentiation, organization, and activity to occur (Chen, Zhang, Mendoza, and Den Besten, 2009). Enamel which is produced under hypocalcemic conditions was found to be reduced in thickness and improperly mineralized (Bonucci, Lozupone, Silvestrini, Favia, and Mocetti, 1994; Chen et al., 2009; Nanci et al., 2000). These outcomes are similar to findings from research examining the effects that deficiencies in other vitamins and minerals have on tooth formation in animals, including boron (Haro Durand, Mesones, Nielsen, and Gorustovich, 2010), vitamin D (Limeback et al., 1992), and vitamin C (Shrestha, More, Keshwar, Shrestha, and Raut, 2019). These deficiencies would be expected in individuals whose diets are low in fruits and dairy products, such as milk and cheese, as well as those with limited sun exposure.

Studies have also assessed the effects of illness and disease on tooth formation. In individuals suffering from inflammatory diseases and infections, their bodies react through the immune system by inducing fever as a protective mechanism (Evans, Repasky, and Fisher, 2015). While the intent of this adaptive response is to increase the performance of immune cells, fever has also been shown to alter enamel formation. In vitro research using experimental animal models has demonstrated that experiencing

high fever alters the activation of gene expression responsible for ameloblast activity (Ryyanen et al., 2014; Tung et al., 2006). This adversely impacts tooth development by impairing enamel formation, due to a reduction in secretion and defective mineralization of enamel matrix (Ryyanen et al., 2014; Tung et al., 2006).

In addition to these clinical studies, anthropological research has explored the impact that biocultural factors in an individual's growth environment have on dental development. These studies are often comparative in nature, evaluating an individual's chronological age relative to their stages of dental and skeletal development (Cardoso, 2007; Conceição and Cardoso, 2011; Kvaal and Haugen, 2017; May, Goodman, and Meindl, 1993; Šešelj, 2013). This is accomplished by assessing discrepancies between age estimations based on attained dental development against those which are based on attained skeletal development, in relationship to known chronological age. The purpose of these studies was to determine if one of these developmental systems shows lesser or greater sensitivity to environmental conditions than the other, with the intent being to further our understanding of developmental plasticity in early life. The existing body of research has established that dental development is more resilient to the adverse effects of stress and negative environmental conditions than bone growth (Cardoso, 2007; Conceição and Cardoso, 2011; Garn, Lewis, and Blizzard, 1965; Lewis and Garn, 1960; Šešelj, 2013; Ubelaker, 1987). These findings have had a substantial impact on how research is conducted in bioarchaeological contexts. The greater sensitivity of skeletal development has led to the use of skeletal morphometrics as indicators of an individual's growth status, which in turn is interpreted as evidence of their lived experiences and biocultural environment (Bleuze, Wheeler, and Williams, 2023). Conversely, the relatively greater robusticity of dental development has led to measurements of an individual's dental development being the preferred method for estimating age, as they have been found to be more accurate than those based on skeletal development (Cardoso, 2007a; Ubelaker, 1987; Ubelaker and Khosrowshahi, 2019).

Although the comparative resilience of dental development to fluctuating biocultural conditions continues to be supported by recent research (Spake et al., 2021), studies conducted over the past several decades (Cardoso, 2007a; Conceição and Cardoso, 2010; Esan and Schepartz, 2019; May, Goodman, and Meindl, 1993; Šešelj, 2013) have demonstrated that tooth formation and developmental timelines are not entirely impervious to adverse biocultural factors. Despite this information, confidence in the reliability of age estimations based on dental development remains unquestioned in many anthropological studies. Notably, recognition of potential errors or biases resulting from inaccurate age estimations and subsequent interpretations is missing from recent bioarchaeological research (Blom et al., 2021; Hodson and Gowland, 2020; Newman, Gowland, and Caffey, 2019).

While existing clinical and anthropological research indicates that development of the dentition can be impacted by environmental perturbations, the extent to which the timing and sequence of tooth formation, and therefore age estimations based on the dentition, are altered has yet to be fully evaluated. Given how foundational dental age estimations are in bioanthropology research, it is apparent that it is necessary to assess how these estimations and associated interpretations are impacted by biocultural factors. By evaluating the degree to which the development of the dentition is disrupted by adverse growth conditions, estimation of age and information pertaining to an individual's lived experiences will be more accurately understood. Assessment of how the developing teeth vary in their response to these environmental perturbations will allow for more informed methodological decisions when selecting which teeth to use for age estimations. This in turn will increase our accuracy for the reconstruction of life history from physical remains, which in turn furthers our knowledge of the lives of individuals and populations in the past.

1.2. The Certosa Collection

1.2.1. Bologna in the 19th Century

Prior to the unification of the Italian states in 1861, otherwise known as the *Risorgimento*, political unrest existed across the peninsula of Italy for decades (Kertzer, Koball, and White, 1997). The city of Bologna was no exception, being controlled by French forces from the end of the 18th century up until 1814, when the end of the Napoleonic Wars allowed for the restoration of papal rule (Kertzer and White, 1994). For the next several decades, Bologna experienced turmoil, with constant revolts requiring the intervention of Austrian troops on behalf of the Pope (Kertzer, Koball, and White, 1997). This continued until 1859, when both Austrian and Vatican authorities were expelled from the city and the area was incorporated into the developing independent kingdom of Italy (Kertzer and White, 1994).

The provinces of Italy came together as a single country in March of 1861, resulting in dramatic changes in both the political and social realms during the latter half of the 19th century (Kertzer, Koball, and White, 1997). This triggered a marked shift in the attitudes of the Italian people, who desired a more liberal approach to society, leaving behind many traditional and outdated beliefs (Ipsen, 2006). Widespread reforms occurred throughout the country, ranging from labour laws to educational mandates (Ginnaio, 2011). The new socialist movement focused on improving conditions for Italy's lower classes, beginning with suffrage being extended from the elite to all men (Kertzer, 1987). Included in this campaign was a national law enacted in 1888, which mandated free medical treatment for the poor in each community (Hogan and Kertzer, 1987). Although this did result in some improvements, the required medications were not without cost, and as such many people could not follow through with their prescribed treatment (Hogan and Kertzer, 1987).

It was also during this time that Italy experienced an industrial revolution, with the number of factories, mines, and mills increasing quite drastically. This led to marked changes in agricultural practices at the time, with many farms shifting from manual labour

to the use of mechanized farm equipment (Hogan and Kertzer, 1987). These industrial and agricultural revolutions had quite detrimental effects on living conditions for children and their families. As more men and women moved away from farm work due to the reduction in labour requirements, families began to migrate to urban centres (Hogan and Kertzer, 1987). This movement led to a rapid increase in the population of Bologna, which in turn led to an accompanying increase in the city's pollution levels (Breschi et al., 2011; Hogan and Kertzer, 1985; Kertzer, 1987). The rate of migration to the city from its surrounding areas was too fast for infrastructure expansion to keep up, resulting in overcrowding and inadequate sanitation (Hogan and Kertzer, 1987).

Conditions for the lower socioeconomic classes of Bologna in the late 1800s were extremely poor. The Agrarian Inquiry of 1881 described the houses of the Bolognese working class as "…the most decrepit, the most filthy, the most unhygienic, … The space available is always the minimum possible…" (Hogan and Kertzer, 1987). As there was no shortage of labourers in need of work and housing, the owners of these dwellings had no motivation to improve upon their conditions. In addition to these poor housing conditions, Bolognese poor were subject to the effects of unsanitary drinking water. As was common throughout Italy, the primary source of water was from numerous wells located throughout the city, with municipal censuses indicating there was approximately one well for every eight citizens (Drusiani et al., 2010; Tuttle, 2015). The dangerous and unhygienic nature of this source of water is evident in the frequent cholera epidemics that occurred throughout the 19th century, as well as a severe outbreak of typhoid fever in 1891 (Drusiani et al., 2010; Faggioli, 2006; Tuttle, 2015).

In addition to their cramped and polluted living environment, the working class of Bologna during this period had an extremely poor diet. This is noted in the Agrarian Inquiry of 1881, which described the nutritional level of the labour class living in Bologna as "terrible in every respect" (Hogan and Kertzer, 1987). Historical records document the diet of the city's poor as consisting primarily of grains with low nutritional value (Fornasin, 1998; Hogan and Kertzer, 1987; Livi Bacci, 1990). Their diet was heavily reliant on corn-

based food items, such as polenta, which in addition to being nutritionally lacking, also carried an increased risk of developing pellagra, a nutritional deficiency associated with vitamin B3 (niacin). Individuals with the condition experience symptoms including gastroenteritis, weakness, dermatosis, and dementia, with approximately 13% of hospitalized "pellagrins" dying of the condition during the late 19th century in Italy (Dalla-Zuanna and Rosina, 2011; Ginnaio, 2011; Lavinder, 1909). Archival records for this period indicate that pellagra was particularly prevalent throughout northern Italy, with the regions of Veneto, Lombardy, and Emilia frequently referred to as the "pellagra triangle" (Ginnaio, 2011; Livi Bacci, 1986).

1.2.2. The Certosa Cemetery

Established by the city of Bologna in 1801, the Certosa cemetery was the first monumental cemetery constructed in Italy (Giannelli, 2008). Formerly the site of a Carthusian monastery (1334 – 1797 AD), the gates of the Certosa cemetery are located approximately 2 kilometres outside of the city's historic walls (Belcastro et al., 2017). This was in keeping with the impending Imperial Decree on Burials of 1804, which dictated that all cities must have a municipal cemetery outside of their boundaries (Vidor, 2012). Prior to this decree, it was common practice for bodies to be inhumed in churches or mass graves. Although the Certosa cemetery is still in use to this day, urban growth over the last two centuries has resulted in it now lying within the current city limits of Bologna.

While the Certosa cemetery has undergone frequent renovations over the past two centuries, many of its structures are from the original monastery. The former refectory was repurposed into a burial chamber (*Sala della Pietà* and the *Sala delle Tombe* of 1816), while several of the monastic cells, such as the *Sala delle Catacombe*, were converted into structures with both burial and administrative purposes (Giannelli, 2008). When the cemetery was initially opened in 1801, the *Chiostro* III, alternatively referred to as *Claustro della Cappella* (English translation: Cloister III and Cloister of the Chapel) was the first area used for inhumations (Martorelli, 2011). The largest area of the cemetery, known as the *Chiostro Maggiore* (English translation: the Greater Cloister), was the

primary burial area reserved for the bodies of Bologna's impoverished citizens. These simple burial plots were a guarantee made by the city of Bologna to ensure a proper burial for those who could not afford a permanent plot, exhibiting a dramatic improvement on the previous practice of using mass graves (Martorelli, 2011; Vidor, 2012). Mention of this designation can be found on a map from 1828 of the Certosa cemetery, which references the area as the "field for the common burial of adults" (Zecchi, 1828). Burial plots for children of the city's poor were located within the *Chiostro* III (Martorelli, 2011; Vidor, 2012). These inhumation areas were further divided into subsections, referred to as *recinto*, by age and sex and are represented by an associated letter (Vidor, 2012). Males under the age of 7 years were inhumed in *recinto* A, while females of the same age range were in *recinto* B (Belcastro et al., 2017). Males who were older than 7 years at the time of their death would have been buried in *recinti* C and F, with females buried in *recinti* D and G. Plots in these *recinti* were offered for a period of 10 years (Vidor, 2012). After the 10 years had passed, the individual would be exhumed and transferred to a common ossuary unless their family could afford to purchase a private tomb (Vidor, 2012).

1.2.3. The Certosa Collection

The Certosa Collection comprises individuals from the cemetery of the same name and is one of the largest documented skeletal collections in Europe (Belcastro et al., 2017). It is currently housed at the Museum of Anthropology of Alma Mater Studiorum University of Bologna, under the curation of Dr. Maria Giovanna Belcastro. Its modern origins and associated records make it ideally suited for anthropological research, particularly those studies which focus on methodological testing of age estimation and analyses of human development.

While neither the University nor the cemetery possess official documents regarding the acquisition of the collection, it is known that it is composed of individuals who were exhumed from the Certosa Cemetery in the late 19th and early 20th centuries (Belcastro et al., 2017). The remains were originally collected by Professor Fabio Frassetto, who was

the Director of the Institute of Archaeology at the University during the first half of the 20th century, and completed by his successor Professor Elsa Graffi Benassi. All of the individuals within the Certosa Collection were buried in the cemetery's simple burial plots within the *recinti* reserved for the city's poor. As previously described, this type of plot was only provided for a period of 10 years, after which the individual would be exhumed. For those individuals whose families were unable to or opted not to purchase a private crypt, their remains would traditionally be moved to a common ossuary (Belcastro et al., 2017). It is likely that the families of the individuals within the Certosa Collection could not afford the cost of a private tomb, which resulted in their remains being relocated to the University of Bologna's Museum of Anthropology instead of being transferred to the common ossuary (Belcastro et al., 2017).

The identities of the individuals within the Certosa Collection are known from associated cemetery records, which provide information pertaining to the individual's name, sex, age at death, occupation, as well as their date and cause of death. These records were retrieved according to the burial number inscribed on a small lead medallion, or "burial badge", which was recovered during exhumation and stored with the remains. These badges were tied around the neck of the individual prior to burial, according to Health and Administration Regulations of the Certosa Cemetery ('Discipline di Sanità pel Cimitero Comunale di Bologna, 1801'; cf. Vidor, 2012, pp. 87–88). Included on the other side of these medallions was a letter indicating which inhumation area, or *recinto*, within the cemetery that the individual was buried (Belcastro et al., 2017). In many instances, the information on the individual's identity was incomplete or difficult to discern, so verification of cemetery and municipal archive records was completed by staff at the Laboratory of Bioarchaeology and Forensic osteology, led by Professor Benedetta Bonfiglioli, in the spring of 2015 (Pedrosi, 2016). In addition to confirming existing data, this endeavour also resulted in the obtainment of information on their place of birth and the identity of their parents. In 2017, Dr. Hugo Cardoso and colleagues obtained birth records from the state archives for most of the juvenile portion of the collection. These records provide the exact date of birth for these individuals, allowing for a determination

of precise age at death, which permits more accurate analyses of skeletal and dental development. Further archival research was completed in 2019, where baptismal records were reviewed at the Archivio Generale Arcivescovile di Bologna, the main repository of the Archbishop of Bologna. These records confirmed the dates of birth and provided addresses for the children's families. In addition, the records listed the birth parish for the children, from which additional historical documents pertaining to their families may be obtained for future research.

There is a total of 425 individuals in the collection (Table 1.1), ranging in age from birth to 91 years old, with males and females being relatively evenly represented (54.8% male and 45.2% female) (Belcastro et al., 2017). Most of the individuals are from the Emilia-Romagna region of Italy, and were born between 1814 and 1922, with years of death ranging from 1898 to 1944. Of importance to this research is the juvenile portion of the collection (age at death ≤ 17 years), which makes up approximately 35% of the total individuals (Table 1.2). The sex distribution remains fairly equal in this category (53.2% male and 46.7% female) and is mostly consistent across this age range (Belcastro et al., 2017). As is expected based on mortality patterns in children, the large majority of these individuals died within the first years of life, with approximately 77% (N = 107) of them being 2 years or younger at their time of death. All of the juvenile individuals within the collection were born after the year 1883 and died in either 1900 or 1901. Due to the lack of documents describing the acquisition of the collection, it is unknown for certain why all of the juvenile individuals are from these two years. A possible theory is that these *recinti* were scheduled for exhumation at the time Prof. Frassetto was assembling the collection from the Certosa Cemetery to make space available for new burials (Mariotti et al., 2015).

TABLE 1.1. The age and sex composition of the Certosa Collection. *Mean and standard deviation in months. **2 infants and 19 adults. Data from Belcastro et al. 2017.

Composition of the Certosa Collection

	Total				Male				Female			
Age at death (years)	N	%	Mean	SD	N	%	Mean	SD	N	%	Mean	SD
< 1*	66	16.6	3.8	4.3	36	16.7	2.3	3.5	30	16.6	5.6	4.6
1 – 9	64	16.1	2.6	2.3	31	14.4	2.4	2.1	33	18.2	2.8	2.4
10 - 19	19	4.8	16.6	2.5	13	6.0	16.2	2.9	6	3.3	17.5	0.8
20 - 29	49	12.3	24.6	3.0	25	11.6	24.0	3.0	24	13.3	25.2	2.9
30 - 39	40	10.1	34.5	3.1	18	8.3	34.6	3.1	22	12.2	34.4	3.1
40 - 49	29	7.3	44.3	2.7	16	7.4	44.3	2.7	13	7.2	44.4	2.8
50 - 59	40	10.1	55.3	3.1	27	12.5	55.3	3.0	13	7.2	55.5	3.3
60 - 69	41	10.3	65.0	2.6	23	10.6	65.3	3.0	18	9.9	64.6	2.1
70 - 79	35	8.8	74.1	2.5	22	10.2	74.1	2.8	13	7.2	74.1	1.9
> 80	1	3.5	83.3	3.6	5	2.3	85.4	4.7	9	5.0	82.1	2.5
≤ 17	139	35.0	2.1	3.9	74	34.3	2.3	4.3	65	35.9	1.9	3.4
18 +	258	65.0	49.2	19.7	142	65.7	50.0	19.6	116	64.1	48.3	20.0
Known age	397	95.0	32.7	27.6	216	94.3	33.7	27.8	181	95.8	31.6	27.5
Unknown age	21**	5.0	-	-	13	5.7	-	-	8	4.2	-	-
Total	418		-	-	229	54.8	-	-	189	45.2	-	-

TABLE 1.2. The age and sex composition of the juvenile segment of the Certosa Collection. Data from Belcastro et al. 2017.

Composition of the Juvenile Segment

	Total		Male		Female	
Age at death (years)	N	%	N	%	N	%
< 1	66	47.5	36	48.6	30	46.2
1 - 2	41	29.5	20	27.0	21	32.3
3 - 4	10	7.2	7	9.5	3	4.6
5 - 6	6	4.3	1	1.4	5	7.7
7 - 8	5	3.6	2	2.7	3	4.5
9 - 10	2	1.4	1	1.4	1	1.5
11 - 12	2	1.4	2	2.7	0	-
13 - 14	1	0.7	1	1.4	0	-
15 - 16	4	2.9	3	4.1	1	1.5
17	2	1.4	1	1.4	1	1.5
Total	139	-	74	53.2	65	46.7

Both the juvenile and adult members of the collection are identified as being of low socioeconomic status (Belcastro et al., 2017). This classification was initially based on their inhumation within the areas of the Certosa Cemetery traditionally reserved for

Bologna's poorer classes – *recinti* A-F in the *Chiostro* III and *Chiostro Maggiore* (Vidor, 2012). For many of the adult members of the collection, as well as a handful of those in their late teens, their low socioeconomic status is supported by the occupation listed on their death certificates. Occupational information is also available for the parents of the children within the collection from their birth records. Very few of the individuals had what would qualify as a skilled job, with most men showing employment as labourers, while the most commonly cited occupation for the women was "housewife" (Belcastro et al., 2017).

To date, research using the Certosa collection has had two main focuses: assessing changes in bone morphology related to early childhood locomotion and improving osteobiographic reconstruction methods. Favoured for its excellent preservation and cross-sectional data throughout human life history stages, the collection offers the opportunity to study how skeletal structures relate to changes in movement (Colombo et al., 2019; Figus et al., 2023; Pietrobelli et al., 2022; Sorrentino et al., 2020), while the known age and sex of the individuals allow for tests of existing approaches to estimation of demographic variables (Marino et al., 2020; Milella et al., 2021; Pietrobelli et al., 2022; Pozzi et al., 2002; Sorrentino et al., 2020; Viciano et al., 2020). This dissertation takes a more biocultural approach to the study of these individuals, exploring the impacts that poor socioeconomic conditions had on their growth and development throughout periods of childhood and across developmental systems. By examining physical evidence of experiences within their socio-historical context, it is intended to expand current knowledge of the children whose remains make up the Certosa collection. It is hoped that this thesis will shed light on what life was like for the children and families of Bologna's poorest class at the turn of the 20th century, in addition to adding to our overall understanding of how ontogenetic processes are impacted by biocultural factors experienced during early life.

2. Exploring the Laws of Developmental Direction using a Documented Skeletal Collection

2.1. Abstract

OBJECTIVE: Many human growth studies have noted a trend of differential variation in limb segment lengths, where more distally situated bones show greater variability relative to those nearer the axial skeleton. It has been proposed that this varying sensitivity stems from the sequence of development, where bones further from the head develop later and are more susceptible to fluctuations in growth conditions. The present study aimed to explore limb dimensions within this theoretical framework, known as the *laws of developmental direction*, using diaphyseal lengths of 46 children (0-11 years) from a documented skeletal collection of low socioeconomic status.

METHODS: Using existing growth standards, z-scores were generated for diaphyseal length measurements of six limb bones. Stunted growth was defined as a z-score of ≤-2. Differences between mean z-score values of the upper and lower limbs, as well as of the proximal and distal segments of each limb, were assessed using paired samples *t*-tests.

RESULTS: The lower limb was significantly more stunted in growth relative to the upper limb (p=<0.001). Similar results were seen within the upper limb, where the distal segment was found to be significantly more stunted in growth relative to the proximal segment (p=<0.001). In contrast, the distal segment of the lower limb was significantly less stunted in growth relative to the proximal segment (p=<0.001).

CONCLUSION: Study findings of increased sensitivity in the lower limb relative to the upper limb and in the distal segment of the upper limb relative to its proximal segment are consistent with the *laws of developmental direction*. However, the finding of greater sensitivity in the proximal bone of the lower limb when compared to both bones of the distal segment does not align with the theorized developmental gradient. These results reveal the complexity of human growth and developmental plasticity in response to biocultural factors.

2.2. Introduction

Organisms respond to environmental stress through developmental plasticity, where the growth of critical organs are prioritized over the development of less vital tissues (Barker, 2012; Bogin and Varela-Silva, 2010; Temple, 2014). This adaptive response has been suggested as the mechanism behind diminished skeletal growth when an individual experiences deleterious developmental conditions, such as malnutrition and disease. As a result of this plasticity, the human skeleton provides a record of exposures to biocultural stressors, enabling reconstructions of the biocultural environment of individuals from their physical remains.

Many studies (Bogin et al., 2002; Holliday, 1999; Higgins and Ruff, 2011; Jantz and Jantz, 1999; Meadows and Jantz, 1995; Tanner, 1978) which have examined skeletal growth in both living and past populations have observed a trend of differential variation in the length of limb segments, where the distal segment of both the upper and lower limbs tends to be more variable relative to the proximal segment. In particular, the length of the tibia is frequently found to be the most variable of all long bones (Auerbach and Sylvester, 2011; Bogin et al., 2002; Meadows and Jantz, 1995; Schweich, 2000; Ulijaszek, 1998). This has been interpreted as evidence that the tibia is more sensitive to biocultural stressors than other long bones (Cardoso and Magalhaes, 2011; Gowland, 2015; Jantz and Jantz, 1999). It has also been suggested that this increased sensitivity extends to the entirety of the lower limb, with studies of modern samples demonstrating greater variability in the length of the leg relative to that of the arm (Bogin et al, 2002; Bogin and Varela-Silva, 2010; Buschang, 1982; Frisancho, 2007; Li, Dangour, and Power, 2007; Jantz and Jantz, 1999; Tanner et al., 1982). Some studies prioritize the use of tibial measurements over other long bones, based on the rationale that this data has the highest potential for detecting growth disruption (DeWitte, 2018; Lampl, Kuzawa, and Jeanty, 2003; Mensforth, 1985; Newman, Gowland, and Caffell, 2019; Pomeroy et al., 2014).

Although the reasons behind this differential sensitivity remain unclear, there have been several explanations proposed, including reduced blood flow (Pomeroy et al., 2012; Holliday and Ruff, 2001 Bogin and Varela-Silva, 2010), thermal regulation (Holliday and Ruff, 2001; Temple et al., 2008; Pomeroy et al., 2012), and the cephalo-caudal principle of growth (Bogin and Varela-Silva, 2010; Schweich, 2000; Jantz and Owsley, 1984). This latter hypothesis is frequently cited in studies focusing on childhood health, where the observed developmental variability is attributed to the differing tempos of growth in the parts of the body (Seeman, 1997; Smith and Buschang, 2005; Bogin and Varela-Silva, 2010). Along with all other mammals, the growth of the human body follows a cephalo-caudal sequence, where those tissues located at the superior or cranial end develop in advance of those located toward the 'tail' (Kingsbury, 1924). A similar gradient is observed in the growth of the appendicular skeleton, which develops in a proximodistal sequence (Scammon, 1923). Together, these two developmental trends have been termed the *laws of developmental direction* (Kingsbury, 1924; Scammon, 1923). As a result, those portions of the body located nearest to the head and trunk are farther along in their development at birth in relation to final adult size (Humphrey, 1998). Elements located farthest from the head and trunk are less developed at birth, and experience more rapid post-natal growth as a result (Kingsbury, 1924). It is widely accepted that those skeletal elements undergoing the fastest rate of growth are more sensitive to environmental perturbations (Cardoso and Magalhaes, 2011; Bogin, 1999; Eveleth and Tanner, 1990; Smith and Buschang, 2004). As the bones of the leg undergo growth more rapidly than those in the upper limb (Buschang, 1982; Cameron, Tanner, and Whitehouse, 1982; Jungers, Cole, and Owsley, 1988; Smith and Buschang, 2005; Bogin and Varela-Silva, 2003), they are more affected by deleterious growth conditions during infancy and childhood. This is also true of the distal limb segments, which experience a faster rate of growth relative to the proximal limb segments (Schweich, 2000; Buschang, 1982; Cameron, Tanner, and Whitehouse, 1982; Smith and Buschang, 2005).

This study aims to explore limb length within the theoretical framework of the *laws of developmental direction*, using a documented skeletal collection of known socioeconomic status. Dating to the turn of the 20th century, the Certosa collection consists of individuals

known to be from the less advantaged social classes of Bologna, Italy (Belcastro et al., 2017). In addition to their impoverished background, the collection is well-suited for research of this nature as the chronological age and sex of each individual is documented from associated birth and death records, permitting precise comparisons of attained skeletal growth for age with available reference standards. These comparisons facilitate the detection of growth disturbances, with a delay in bone growth reflected as a negative discrepancy between the diaphyseal length of sample individuals relative to the mean length for age from a reference standard.

If those bones located farthest from the trunk are more sensitive to those biocultural insults which affect growth, it is expected to see greater growth delays in the lengths of the bones of the distal limb segment (the tibia/fibula and radius/ulna) relative to the proximal limb segment (the femur and humerus). Given the faster growth of the lower limb versus the upper limb, it is also expected that there will be greater growth delays in the lengths of the bones of the leg relative to those of the arm. The main goals of this study are, first, to examine evidence of differential growth in limb lengths to test if greater relative sensitivity to poor growth conditions is observed in the lower limb within the study sample. This is evaluated through comparisons of diaphyseal length of the bones of the lower limb (the femur, tibia, and fibula) relative to those of the upper limb (the humerus, radius, and ulna). Second, growth stunting in the distal limb segments will be evaluated in relation to the proximal limb segments.

By evaluating the growth of these individuals in the context of the *laws of developmental direction*, it is intended that a greater understanding of how various environmental stressors, such as disease and inadequate nutrition, may be responded to through developmental plasticity and the potential impact this might have on growth of the limb segments. These interpretations of the biocultural growth environment will be strengthened by the age and sex comparisons made possible through the archival documentation of each child from this stratum of late 19th century Bolognese society. It is anticipated that through this research, an

increased understanding of how to accurately interpret information pertaining to an individual's health and biocultural environment from their skeletal remains will be achieved.

2.3. Materials and Methods

2.3.1. Sample

The study sample was selected from the identified skeletal assemblage of known age and sex individuals amassed from the Certosa Cemetery in Bologna, a large city located in the north of Italy. The collection was created for research and teaching purposes in the mid 1900s and comprises 425 individuals who lived and died in the late 19th to early 20th centuries (Belcastro et al., 2017). These individuals range in age from birth to 91 years of age at the time of their death, with approximately equal numbers of male (55%) and females (45%) represented. Based on their inhumation in those areas of the Certosa Cemetery reserved for impoverished individuals (Vidor, 2012), the collection is taken as representing Bologna's poorest social class. Archival research of civil and church records, housed at the Archivio di Stato di Bologna and Archivio Generale Arcivescovile di Bologna respectively, conducted by the authors in the past several years has supported the assessment of these individuals being from an impoverished socioeconomic background. These documents included both birth and baptismal documents, where many of the adult males in the collection were recorded as being employed as unskilled labourers, while the occupation for the majority of females is listed as housewife (Belcastro et al., 2017).

This study examines those individuals in the collection documented as under the age of 12 years according to their death record. The age and sex of the Certosa children are determined from archival documents, including birth, death, and baptism records. These documents permitted the calculation of chronological age at death to the nearest day for all but five individuals, whose age at death could be determined only to the nearest year. The decision to include only those children younger than 12 years of age was made to ensure comparability between the study sample and the long bone reference length data by including only children with unfused long bone epiphyses. Individuals where damage or

breakage may have impacted accurate measurement of diaphyseal length were excluded from the study, as were individuals with obvious skeletal pathological conditions, such as rickets. Forty-six individuals from the Certosa Collection were included in this study. Males and females were evaluated using sex-specific reference data according to their sex as listed on associated archival records. Chronological age was analysed in years by conversion to decimal age from birth and death dates. Table 2.1 provides the age and sex composition of the study sample.

TABLE 2.1. Age and sex composition of the study sample.

Age (years)	Total	Female	Male
0	3	2	1
0.5	8	7	1
1	13	8	5
2	4	2	2
3	4	1	3
4	1	1	0
5	5	4	1
6	1	1	0
7	2	1	1
8	0	0	0
9	2	1	1
10	0	0	0
11	2	0	2

2.3.2. Skeletal Data

The maximum diaphyseal lengths of the humerus, radius, ulna, femur, tibia, and fibula were measured to the nearest tenth of a millimeter using digital sliding calipers, or to the nearest millimeter with a standard osteometric board, following accepted protocols (Buikstra and

Ubelaker, 1994). The left side was used when possible, with the right accepted as a substitute in instances of damaged or missing bones. Summary statistics for diaphyseal length were calculated for all long bones using one-year intervals for individuals over one year of age, while those under this age were calculated using half year intervals. Sexes were pooled in order to provide comparable data with studies of childhood growth (Mays, 1999; Ruff et al., 2013), where biological sex is often unknown. As most body size differences between sexes are due to changes linked to puberty, a pre-pubertal sample such as the one in this study is not likely to be significantly altered in the reported statistics, but rather benefits from the bolstered sample size.

Diaphyseal length data from the study sample were compared to reference data drawn from the Denver Child Research Council Study, alternatively known as the Maresh dataset (Maresh, 1943, 1955, 1970; Ruff, 2003; Schillaci et al., 2012). The original Maresh sample comprises 175 children living in the Denver area in the mid-1950s, all of whom were of European ancestry and primarily of a middle to upper middle-class background (Maresh, 1943, 1955). Individuals were examined and radiographed at regular intervals during development, resulting in a database of nearly 700 radiographs documenting diaphyseal growth between 2 months and 12 years of age. These data are widely accepted as being representative of a normal human growth pattern and are frequently used for comparative analyses of long bone growth in archaeological contexts (Schillaci et al., 2012). Recently, these data were updated by Spake and Cardoso (2021), who corrected the measurements for radiographic magnification and adapted the values using polynomial regression to calculate smaller age cohorts of 1 month, permitting more refined analyses. Spake and Cardoso's (2021) values were used as a comparative reference for diaphyseal length in the present study.

2.3.3. Statistical Analyses

Measurement error in diaphyseal length was assessed through observer error tests, where a sample of 20 bones was measured by one observer, and then re-measured by the first

observer and a second observer after a period of two weeks. The relative technical error of measurement (%TEM) and coefficient of reliability (*R*) were calculated following the method established by Ulijaszek and Kerr (1999). A single calculation of %TEM and *R* was used to compare observations and assess intra- and inter-observer error.

Differences in limb length for age between the study sample and reference data were assessed through z-score calculations. Comparisons between the study sample and the reference population were done using 1-month intervals, using the age values for the reference sample calculated by Spake and Cardoso (2021). Individuals were assigned to the last attained age threshold. Z-score analysis was employed to standardize the differences in the diaphyseal lengths of the Certosa children and the age-specific means from the reference data. Z-scores are commonly used in both clinical and biological studies of living children to assess growth, as they provide a quantifiable method of comparing differences within and between groups (WHO, 1995). A z-score provides a measure of how many standard deviations (SD) the value from an individual deviates from the expected mean value of the age- and sex-specific reference standard, calculated as $z = (X - \mu) / \sigma$ where X is the diaphyseal length measurement of the study individual, μ is the mean diaphyseal length from the reference sample, and σ is the standard deviation of the diaphyseal length from the reference population. For studies assessing growth in children, a z-score of -2 or lower is defined as stunted (WHO, 1995). Z-scores were calculated for each long bone diaphysis. The z-score values for the lower limb were calculated as an average of the proximal and distal values [(femur + (tibia + fibula)/2)/2], while upper limb was calculated as an average of the proximal and distal values [(humerus + (ulna + radius)/2)/2]. In addition, a composite z-score statistic (CZS) was calculated as the mean of all diaphyseal length z-scores for each individual.

Differences between the diaphyseal lengths of the study sample and reference population were assessed through the calculation of long bone length for age z-scores, which provide a measure of growth faltering controlled for age and sex. Paired samples *t*-tests were used to assess the significance of differences between the mean z-score values of the upper and

lower limbs, as well as between the proximal and distal segments of each limb. All tests were done with an alpha level of 0.05. Z-score results between the males and females of the collection were assessed for significance of difference using an independent samples *t*-test. The decision to use the *t*-test was based on the results of the Shapiro-Wilk test of normality and Breusch-Pagan test of heteroscedasticity. All statistical procedures were performed using the statistical packages jmv and moretests in jamovi (Version 2.2; The jamovi project, 2021).

To determine if differences in diaphyseal long bone lengths between the study sample and reference data, as well as patterns of growth faltering, are attributable to the appropriateness of the Maresh dataset as a reference standard, comparative analyses were conducted using an alternative dataset, derived from children within the Lisbon, Spitalfields, and St. Bride's Church skeletal collections (Cardoso et al., 2014). These individuals were all of European descent and are considered to be from socioculturally comparable contexts. As there are no other datasets which provide mean length measurements for all six unfused long bones, it was not possible to calculate all necessary z-scores as a method of comparison. Using age prediction formulae developed by Cardoso and colleagues (2014), the expected diaphyseal length based on known age was calculated by reversing the terms of the classical calibration equations for the estimation of age. These formulae were developed for the purpose of estimating age based on long bone development for a wide range of populations, but are particularly applicable for populations of a similar time period and region, such as the children of the Certosa collection. As the long bone lengths from the study sample fall within the range suitable for the age estimation formulae, they were considered to serve as an appropriate standard of comparison. The sign and magnitude of discrepancies between actual length and predicted length were compared and used to detect diminished growth in the bones of the sample individuals. These results were then compared to those produced using the Maresh dataset to assess for consistency in the study findings.

2.4. Results

2.4.1. Tests of Measurement Error

Results of the intra-observer and inter-observer error tests indicate that less than 1% of variation in the sample data is attributable to observer error (Table 2.2).

TABLE 2.2. Intra- and inter-observer error test results for diaphyseal length measurements.

Intra-observer		Inter-observer	
%TEM	*R*	%TEM	*R*
2.16	0.998	2.08	0.998

Abbreviations: %TEM, relative technical error of measurement; R, coefficient of reliability.

2.4.2. Skeletal Growth Analysis

Summary statistics for diaphyseal length are included in Table 2.3. Data for each long bone are presented separately, with the sexes pooled and the sample broken down into one year age intervals (1 = 1.0 – 1.9 years of age), save for those individuals under the age of one year, who are presented in half year intervals. Sex specific summary statistics are provided in Appendices A and B.

TABLE 2.3. Summary statistics of diaphyseal length (mm) for each long bone (sexes pooled).

Age	Humerus			Radius			Ulna		
(years)	n	x̄	SD	n	x̄	SD	n	x̄	SD
0	3	70.35	2.84	3	53.65	1.79	2	60.44	0.85
0.5	7	83.70	8.57	7	62.76	4.88	5	73.85	3.62
1	12	107.08	11.44	8	77.57	11.15	7	87.44	11.07
2	4	117.56	9.57	3	87.84	8.62	3	96.76	11.03
3	3	136.75	3.04	1	101.54	/	2	108.31	0.40
4	1	156.44	/	1	109.57	/	1	121.22	/
5	4	155.77	11.68	4	112.96	10.45	4	123.19	11.86
6	1	171.77	/	1	122.2	/	1	131.45	/
7	2	190.57	3.44	2	137.97	5.01	2	149.35	5.17
8	/	/	/	/	/	/	/	/	/
9	2	191.41	15.68	2	140.86	16.36	2	152.91	13.35
10	/	/	/	/	/	/	/	/	/
11	2	214.29	9.49	2	150.59	6.94	2	166.02	10.58

Age	Femur			Tibia			Fibula		
(years)	n	x̄	SD	n	x̄	SD	n	x̄	SD
0	3	81.36	4.63	3	68.77	4.14	3	65.70	4.53
0.5	9	107.11	13.55	8	89.88	11.17	6	87.61	9.46
1	13	132.30	13.90	13	109.18	12.28	9	106.38	15.14
2	4	150.32	16.26	4	121.96	14.66	4	117.11	12.99
3	4	172.78	3.23	3	143.63	4.07	2	138.2	2.23
4	1	206.50	/	1	206.50	/	1	164.47	/
5	5	216.40	11.25	5	174.28	10.25	5	170.73	9.60
6	1	246.00	/	1	191.43	/	1	187.71	/
7	2	277.25	4.60	2	224.25	7.42	2	211.25	13.08
8	/	/	/	/	/	/	/	/	/
9	2	255.00	19.80	2	210.75	20.86	2	208.50	22.63
10	/	/	/	/	/	/	/	/	/
11	2	283.50	2.83	2	233.25	12.37	2	230.00	9.19

2.4.3. Inter-Sample Comparisons

The results of the z-score summary statistics for the sample presented separately by bone (Table 2.4) show a difference in diaphyseal lengths between the study sample and the reference population, with the Certosa children exhibiting a delay in skeletal growth relative to the updated Maresh data (Spake and Cardoso, 2021). Diaphyseal lengths from the sample individuals show a substantial growth deficit relative to the reference population with a mean z-score= -2.00 which is the globally recognized value for stunted growth (WHO, 1995). The children in the sample population are smaller than the reference individuals in all diaphyseal lengths, with 50% (n=23) of individuals producing a composite z-score (CZS) value ≤ -2. Analysis of the sample distribution for CZS values demonstrates that very few children in the study sample have positive values (Figure 1.1.). The difference between CZS values and zero was shown to be significant using a one sample *t*-test (p = <0.001).

TABLE 2.4. Long bone z-scores summary statistics.

Bone	n	x̄	Median	SD
Humerus	41	-1.47	-1.50	1.3022
Radius	34	-2.14	-1.93	1.3137
Ulna	33	-2.43	-2.37	1.3133
Femur	46	-2.45	-2.46	1.4439
Tibia	44	-1.81	-1.91	1.3908
Fibula	37	-2.10	-2.22	1.4238
CZS	46	-2.00	-1.96	1.3062

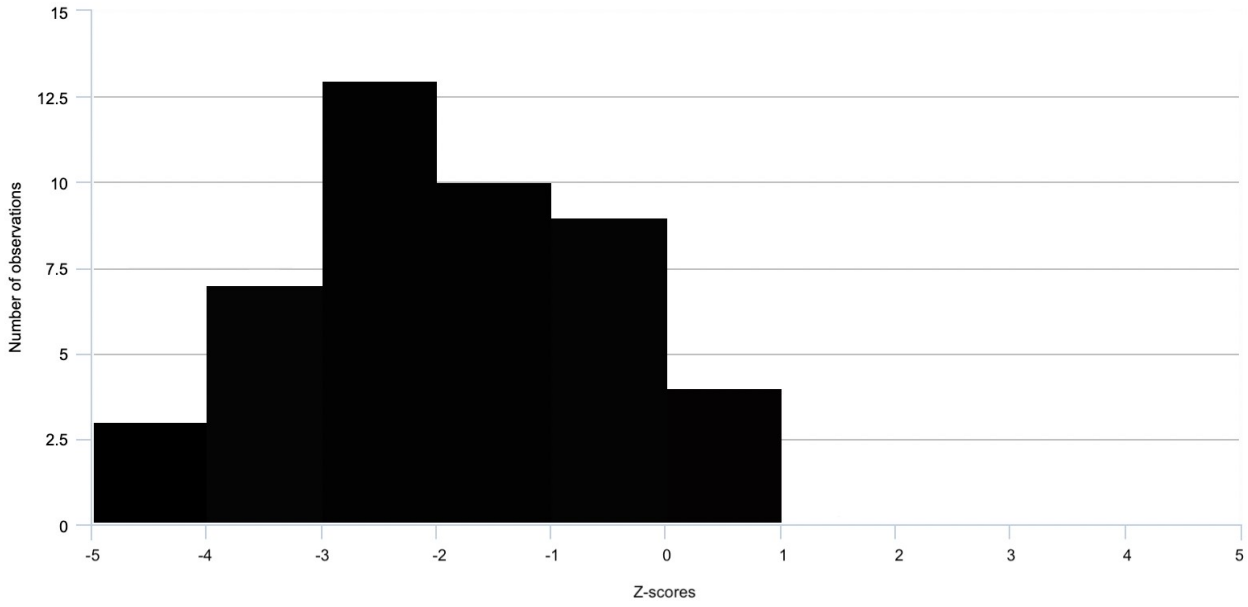

FIGURE 2.1. Distribution of composite z-score values with the sexes pooled (n = 46).

Figure 2.1 shows z-scores for humeral, radial, ulnar, femoral, tibial, and fibular diaphyseal length by age of the Certosa children compared with the reference data. The deficit between sample individuals and the reference group increases with age, with 75% of individuals over the age of 5 years qualifying as stunted (≤ -2) in their CZS, compared to 32% of individuals under the age of 2 years. All of the diaphyseal lengths examined produce a stunted mean z-

score value (≤ -2), except for the humerus (mean z-score = -1.47) and tibia (mean z-score = -1.81). All diaphyseal length means differ significantly ($p<0.01$) from the reference data. Individuals identified as potential outliers were explored. Results do not change when these individuals are removed from analyses.

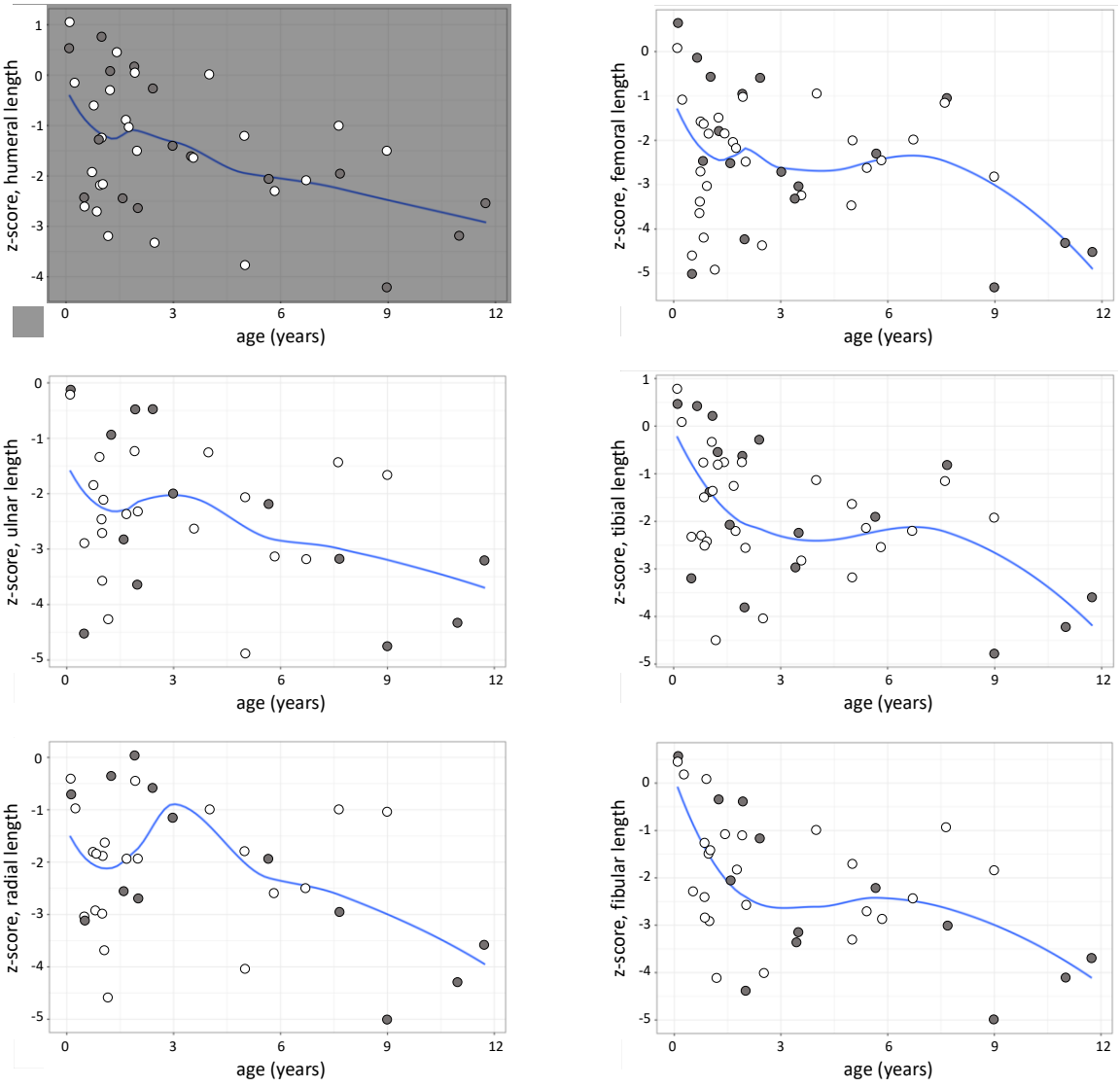

FIGURE 2.2. Loess curve fitted to z-score for age data of diaphyseal lengths compared with the Maresh reference means (represented by the ``0.00 line''). Grey markers represent male individuals, while white markers represent female individuals.

When sex groups are considered separately, it is apparent from mean z-score values (Table 5) that diminished growth is more prevalent in males than in females. Although the males in the study sample produce lower mean z-scores for all long bones, the mean z-score differences between the sexes are not statistically significant for any bone (Table 2.5). The distribution of these differences are demonstrated in Figure 2.3. This result could be an outcome of the greater number of females in the study sample.

TABLE 2.5. Long bone z-scores summary statistics by sex.

	Females				Males				Independent Samples t-test	
Bone	n	$\bar{x}$	Median	SD	n	$\bar{x}$	Median	SD	*t*	*p*
Humerus	25	-1.43	-1.50	1.233	16	-1.53	-1.78	1.440	0.226	0.822
Radius	21	-2.09	-1.87	1.148	13	-2.21	-2.55	1.593	0.265	0.793
Ulna	20	-2.38	-2.34	1.107	13	-2.51	-2.83	1.627	0.285	0.777
Femur	28	-2.45	-2.31	1.220	18	-2.46	-2.49	1.776	0.013	0.990
Tibia	27	-1.78	-1.92	1.188	17	-1.84	-1.90	1.704	0.133	0.895
Fibula	24	-1.89	-1.84	1.214	13	-2.49	-3.02	1.734	1.230	0.228
CZS	28	-1.97	-1.81	1.118	18	-2.04	-2.13	1.589	0.169	0.866

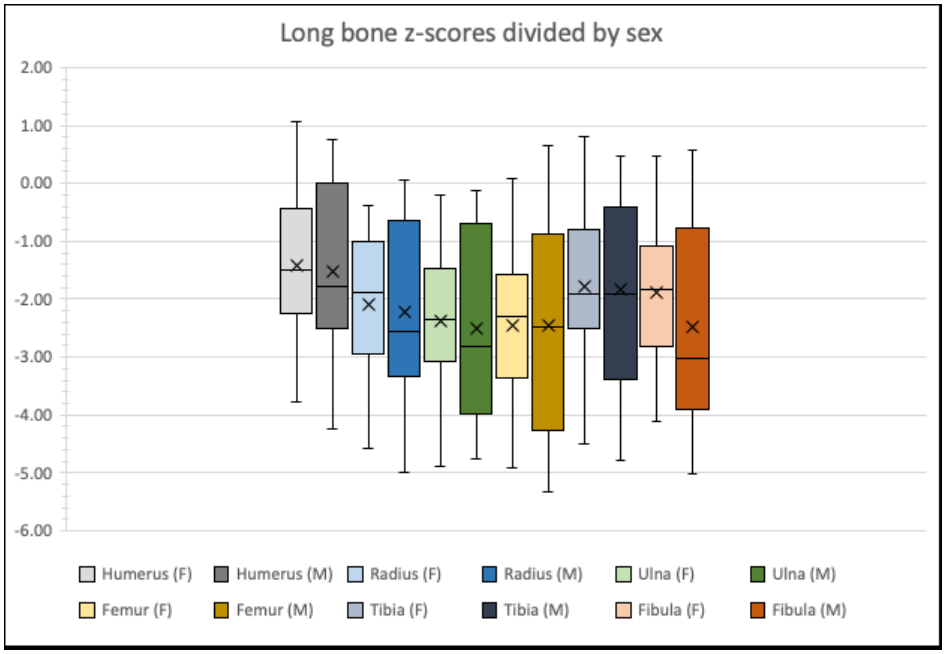

FIGURE 2.3. Box plots for each of the six long bones comparing the distribution of female and male z-scores. The x represents the mean value, while the median is represented by the line.

2.4.4. Intra-Sample Comparisons: Upper vs. Lower Limb

Analysis of the mean z-scores between the upper and lower limbs indicate differences in their growth. The z-score values (Table 2.6) indicate that relative to the reference population, the lower limb has more diminished growth than the upper limb. This is demonstrated in Figure 2.4, where lower limb length is plotted against upper limb length, with a reference line representing the mean values from the reference sample. The majority of the observations are located on or slightly above the reference line, indicating that lower limb length in the study sample tends to be slightly shorter relative to upper limb length. A paired samples t-test indicates the difference between the mean z-score of the upper limb and lower limb is statistically significant ($p = <0.001$), with the lower limb having more diminished growth relative to the upper limb.

TABLE 2.6. Upper and lower limb z-scores summary statistics.

Upper Limb				Lower Limb			
n	x̄	Median	SD	n	x̄	Median	SD
43	-1.74	-1.62	1.3072	46	-2.20	-2.17	1.3852

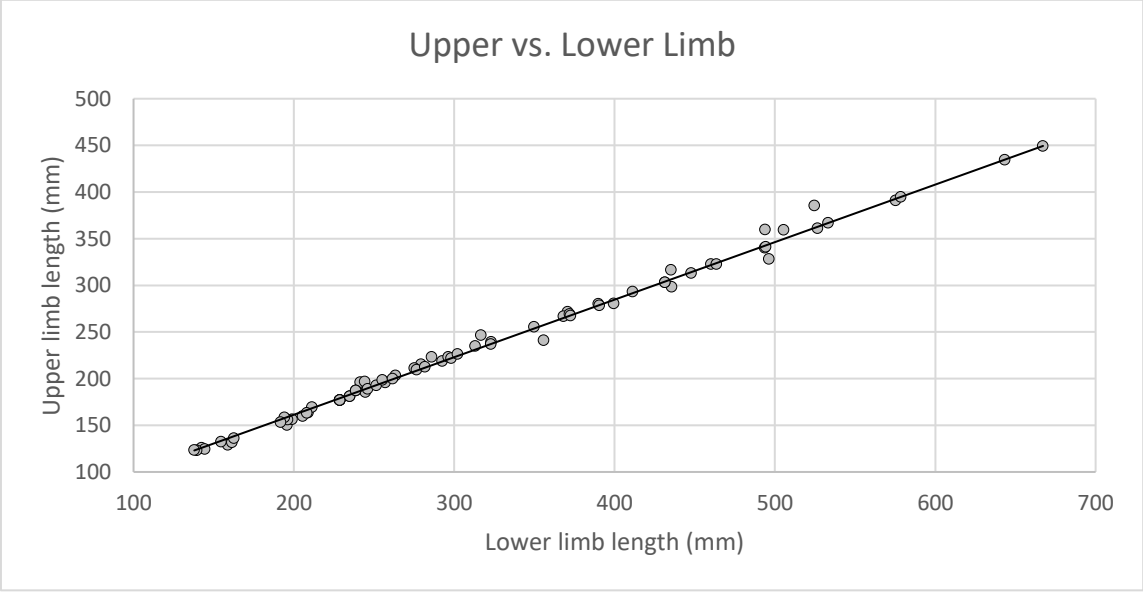

FIGURE 2.4. Lower limb length versus upper limb length (sexes pooled). The line represents the profile of the reference sample (Spake and Cardoso, 2021).

2.4.5. Intra-Sample Comparisons: Proximal vs. Distal Limb Segments

Upper Limb

Comparisons of the mean z-score values of the proximal and distal limb segments demonstrate differing growth between the two. In the upper limb, the mean z-score of the ulna and radius is significantly lower than that of the humerus (Table 2.7), indicating more delayed growth in the distal segment of the arm relative to its proximal segment. This difference is demonstrated in Figure 2.5, which shows the plot of humerus diaphyseal length against the averaged diaphyseal length of the ulna and radius. Nearly all of the observations are below the reference line (Spake and Cardoso, 2021), indicating that the proximal segment in the study sample tends to be longer relative to the distal segment in the upper limb. The difference between mean z-score values for the proximal and distal segments of the upper limb was found to be significant by a paired samples *t*-test (p = <0.001), with the distal segment demonstrating greater growth faltering than the proximal segment in the arm. When the bones of the distal segment are compared separately to the proximal segment, the difference is still found to be significant (ulna, p = <0.001; radius, p = <0.001). This significance persists when the sexes are evaluated separately (males, p = <0.001; females, p = <0.001).

TABLE 2.7. Upper limb z-scores summary statistics.

Distal Segment				Proximal Segment			
n	x̄	Median	SD	n	x̄	Median	SD
35	-2.26	-2.12	1.2785	41	-1.47	-1.50	1.3022

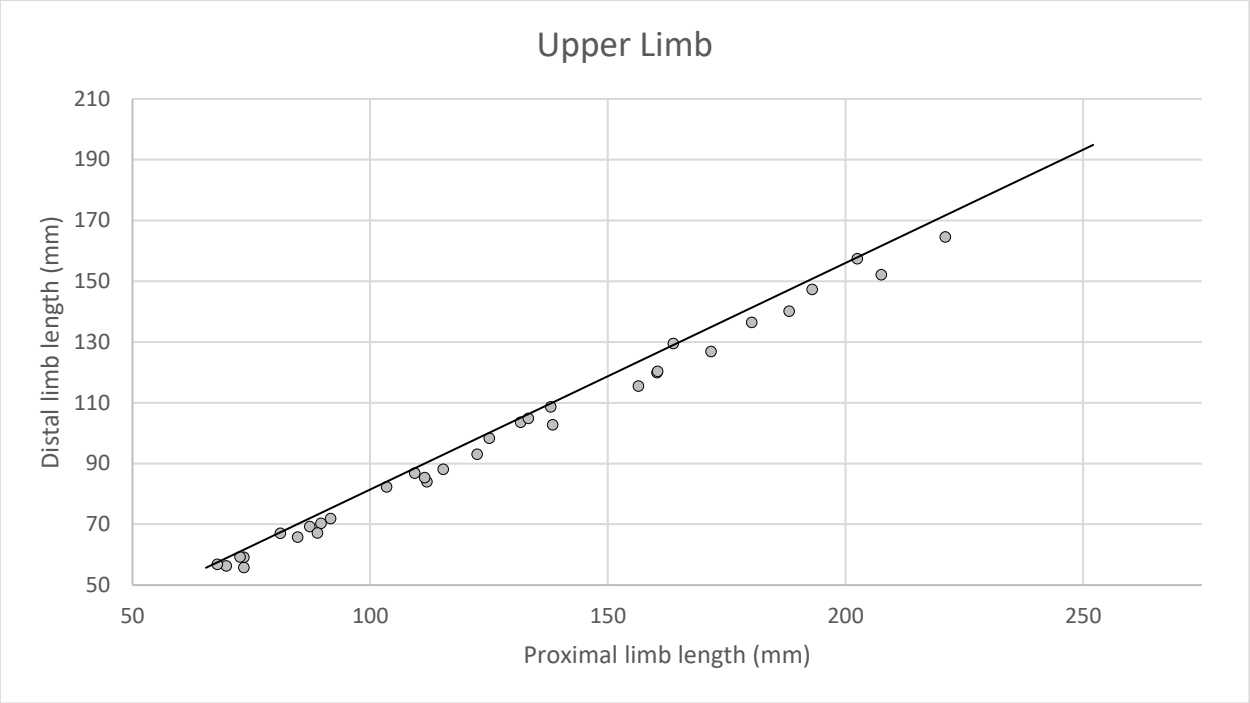

FIGURE 2.5. Distal limb segment length versus proximal limb segment length of the upper limb (sexes pooled). The line represents the profile of the reference sample (Spake and Cardoso, 2021).

Lower Limb

Evaluation of the proximal and distal segments in the lower limb reveal a different trend, with mean z-score values of the femur being lower than those of the tibia and fibula (Table 2.8). This shows that the proximal segment of the lower limb tends to be more delayed in growth relative to the distal segment. When proximal segment length is plotted against the length of the distal segment (Figure 2.6), the majority of the observations are located on or slightly above the reference line (Spake and Cardoso, 2021), indicating that in the study sample, the proximal segment of the lower limb tends to be shorter relative to the distal segment. A paired samples t-test (p = <0.001) revealed the proximal segment of the lower limb was significantly more diminished in growth relative to the distal segment. This significance persists when the bones of the distal segment are compared separately to the

proximal segment (tibia, p = <0.001; fibula, p = <0.014). This significance persists when the sexes are evaluated separately (males, p = <0.008; females, p = <0.001).

TABLE 2.8. Lower limb z-scores summary statistics

Distal Segment				Proximal Segment			
n	x̄	Median	SD	n	x̄	Median	SD
44	-1.86	-1.90	1.3900	46	-2.45	-2.46	1.4439

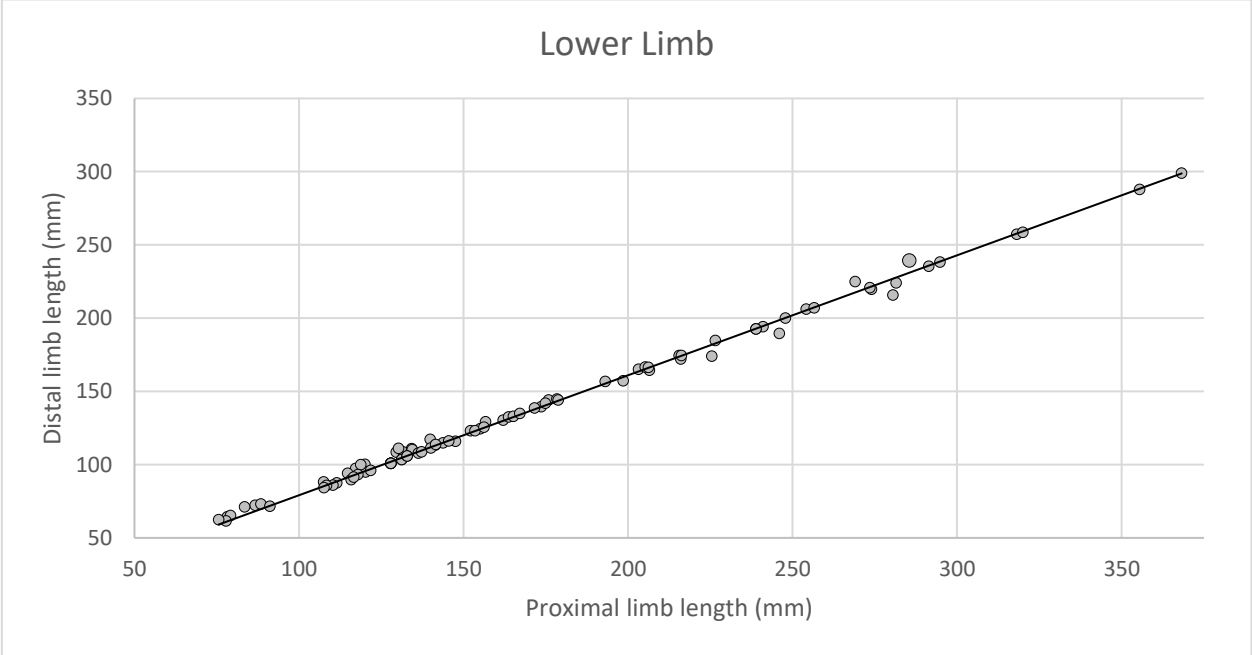

FIGURE 2.6. Distal limb segment length versus proximal limb segment length of the lower limb (sexes pooled). The line represents the profile of the reference sample (Spake and Cardoso, 2021).

Results from the comparative analyses conducted using an alternative dataset from Cardoso and colleagues (2014) are provided in Table 2.9, displayed as the mean discrepancies between expected diaphyseal length based on known age and actual length for each bone. The discrepancy of the humerus (mean = -0.79) was significantly smaller (p = 0.001) than the mean discrepancies of the radius (mean = -2.74) and ulna (mean = -3.35). In the lower limb, the discrepancy of the tibia (mean = 1.22) was significantly different (p ≤ 0.001) than that of

both the femur (mean = -2.58) and fibula (mean = -2.23). These results are consistent with those found using the Denver dataset for z-score calculations.

TABLE 2.9. Discrepancies between predicted length for age and actual length (cm) for each long bone (sexes pooled) using Cardoso et al. (2014) formulae.

Humerus			Radius			Ulna		
n	x̄	SD	n	x̄	SD	n	x̄	SD
41	-0.79	9.12	34	-2.74	8.30	33	-3.35	8.36
Femur			Tibia			Fibula		
n	x̄	SD	n	x̄	SD	n	x̄	SD
46	-2.58	14.52	44	1.22	12.32	37	-2.23	11.95

2.5. Discussion

The children of the Certosa collection demonstrate delayed or diminished linear skeletal growth in comparison to the Denver reference sample, with stunting (=<-2 S.D.) in a number of long bones. It is apparent from study findings that variation exists in the development of the limbs and limb segments in the Certosa children. The lower limb was found to be more delayed in growth relative to the upper limb, and the distal segment of the upper limb exhibited a greater growth deficit than was observed in its proximal segment. These results are consistent with the *laws of developmental direction*, which predict increased sensitivity to deleterious growth environments in those skeletal elements furthest from the head and trunk. In contrast to the upper limb, the bones of the leg display a different pattern of affected growth, where the proximal bone was found to be significantly more delayed in growth in comparison to both bones of the distal segment. This finding does not align with the anticipated study outcomes based on the theorized developmental gradient. The differences in growth delay and deviation from expected findings based on existing literature warrant further consideration in light of the sample context, such as maternal health, documented illness, and nutrition.

Given the impoverished background of the children buried in this section of the cemetery, the finding of diminished growth in this population is not unexpected and aligns with previous research into the collection (Nelson et al., 2020; 2021), which found the vertebral and dental development of these children to be reduced when compared to existing reference standards (Hinck et al., 1962; Hinck et al., 1965; Hinck et al., 1966; Cardoso et al., 2016, 2019). As growth of dental and spinal tissues is considered to be more resilient to environmental perturbations than bones of the appendicular skeleton (Cardoso, 2007; Clark et al., 1986), it is not surprising that the more sensitive diaphyseal long bone lengths of these children also exhibit growth delays.

In examining the prevalence and patterns of diminished growth within the Certosa collection and the possible biocultural factors involved, it is prudent to first discuss issues stemming from the osteological paradox (Wood et al., 1992). As the study sample is composed of children whose biocultural circumstances resulted in their premature death, inherent biases exist when comparatively analyzing their growth against that of living children. It must be acknowledged that the developmental factors which negatively impacted the growth of their skeletal tissues increased the likelihood of them dying, thereby making it more likely that diminished growth will be observed in the study sample than would be seen in a living population. This bias has been dubbed the biological mortality bias (Saunders and Hoppa, 1993). Based on this theoretical framework and the increased probability of stunted children dying (de Onis et al., 2018; McDonald et al., 2013; Thurstans et al., 2022), it is likely that those children from the study sample experienced greater diminished growth, as well as differing growth patterns, than what might be observed in individuals who survived past childhood. This is worth noting, as many studies whose results are supportive of the *laws of developmental direction* were conducted using data from living individuals (Bogin et al., 2002; Buschang, 1982; Frisancho, 2007; Leung et al., 1996; Takamura et al., 1988; Wadsworth et al., 2002). If the biocultural stress or developmental delay was severe enough to be a contributing factor in the individual's death, it may then be severe enough to impact proximally located skeletal elements. This bias could be behind the unexpected finding of the significantly greater stunting observed in the proximal leg segment in the study sample.

While this differs from the pattern of diminished growth typically seen in living children experiencing poor biocultural conditions, how it presents in deceased individuals may vary and indicate more severe developmental concerns.

Further to this point, the biological mortality bias may also be present in the age composition of the study sample via selective morality (Wood et al., 1992). As those individuals in the first years of life experience the greatest mortality, this results in a disproportionately high number of individuals in the youngest age groups, which in turn may impact the prevalence of growth stunting when compared with older individuals. As growth patterns are known to vary with age, where the tempo and timeline for individual skeletal elements do not remain consistent throughout childhood (Harrison, 1992; Cunningham et al., 2017; Sinclair, 1989), this may alter the pattern of affected growth and be a contributing factor to the results found in the study sample. Specifically, as long bone growth rates are known to be most rapid within the first years of life (Humphrey, 1998), this could result in substantially diminished growth or differing patterns of growth stunting in the youngest individuals of the sample. In particular, the femur undergoes relatively more growth during the first years of life than the other long bones (Cunningham et al., 2021). This may in turn lead to a higher level of growth stunting being observed within the study sample relative to what may be experienced in one with more evenly distributed age groups. Without the ability to comparatively assess the longitudinal growth of individuals who survived childhood into adulthood with the growth of those children who prematurely died and are included in this study sample, discussion of potential explanations for these findings and interpretations of early health and development in this population are limited by the paradoxical nature of the data. To mediate the inherent biases present in the interpretation of skeletal data, additional evidence is presented throughout this discussion in the form of historical and archival resources, providing non-biological data for sample contextualization. These include census data pertaining to infant and child mortality from this time period in Northern Italy, as well as statistical records of disease prevalence in the region.

It may be suggested that the unexpected study findings of greater stunting in the proximal segment of the leg are due to issues pertaining to the comparability of the reference dataset and the study sample. Data from the Denver Growth Study has been frequently used in anthropological studies of long bone growth and stature in many populations (Schillaci et al., 2012). It is one of the only studies of its kind, with no others existing that match its completeness for longitudinal data of all six long bones from infancy to adulthood. However, concerns have been raised in regard to the use of the Denver sample as an example of typical or normal child development (Cardoso and Magalhães, 2011; Spake and Cardoso, 2018), with children from the Denver sample being found to be significantly smaller-for-age compared to individuals from other reference samples (Fels and Harvard). While the data has been found to conform to modern WHO standards for stature and are therefore suitable for evaluating both stature and femoral growth patterns of diverse skeletal assemblages, issues have been identified in its use for detecting statural stunting. A 2012 study by Schillaci and colleagues found that when using the Denver dataset to assess stature in a sample of children from an Indian population, the analysis resulted in both underestimation and overestimation of stunting prevalence, with patterns varying based on age and sex (Schillaci et al., 2012). These findings may indicate that individuals in the Denver Growth study were not experiencing unimpeded growth, which in turn could result in varying limb proportions and impact the results of this study in regards to detecting stunting in the sample population. In order to assess if this is the case, a comparative analysis was conducted using published data from the Lisbon, Spitalfields, and St. Bride's skeletal collections as an alternative dataset (Cardoso et al., 2014). Although this analysis differs in its approach to the data (discrepancy between actual length and length predicted based on age vs. z-score calculation), the pattern of results for proximal vs. distal limb segments for the study sample does not differ from that produced using the Maresh sample. The distal segment of the upper limb deviates more from expected length than the proximal segment, while the proximal segment of the lower limb has a greater discrepancy from expected length than either of the distal bones. This indicates that the pattern of differential growth delays seen in the Certosa individuals is not the result of issues with the comparative sample.

The observed variation in the growth of the limbs and limb segments may be indicative of differing exposure to adverse growth conditions during the developmental period. It has been established that skeletal elements undergo development on unique and varying schedules and tempos (Harrison, 1992; Cunningham et al., 2017; Sinclair, 1989), which might then align with varying biocultural factors associated with these developmental periods. As previously mentioned, the differing growth timelines of the various regions of the body is a key component behind the *laws of developmental direction*. The conceptual framework posits that those elements located towards the head and trunk undergo development earlier than the more distally situated bones (Harrison, 1992; Sinclair, 1989; Tanner, 1962). This in turn leads to a greater amount of development occurring *in utero*, which is typically considered to provide a buffered growth environment, where the fetus is protected from potentially adverse biocultural factors they may be exposed to postnatally. The increased sensitivity and stunting of distal elements are often attributed to the relatively greater amount of growth they undergo postnatally, where their exposure to negative biocultural factors and more rapid rate of growth may result in heightened levels of diminished growth (Buschang, 1982; Frisancho, 2007; Martorell, 1989; Tanner, 1962).

When conducting research using archaeological cross-sectional data, such as the present study, it is not possible to ascertain causal factors behind the observed variation in limb growth, however identifying potential contributors is possible. One such factor is that of maternal health status and the role it may play in skeletal development of the child. In instances of poor maternal health, the benefits of greater *in utero* development may not be as pronounced and may even result in those bones undergoing more of their growth during the fetal period experiencing negative growth effects. There are many scholarly articles which delve into the effects that maternal biocultural status, such as malnutrition (Black et al., 2008), smoking (Knopik et al., 2012; Lampl, Kuzawa, and Jeanty, 2003), illness (Lampl and Jeanty, 2004) and psychological stress (Dancause et al., 2012), has on *in utero* growth and the development of the child. The consequences range from underdeveloped organs and low birth weight to miscarriage. Research has also demonstrated a link between childhood stunting and fetal development, indicating a prenatal origin to growth faltering in children of

mothers with poor health (Christian et al., 2003). Although the potential impacts are quite varied, there is substantial evidence for the connection between maternal health and fetal development, demonstrating a clear cause and effect relationship at all stages of the *in utero* period (McMullen and Mostyn, 2009). Clinical research exploring the impact of maternal weight on fetal growth found that the femur experienced a faster rate of growth than the tibia, both in infants born stunted at birth and those who were not stunted at birth (Neufeld et al., 2014). The femur also exhibits comparatively faster rates of prenatal growth when evaluated against the humerus (Mehta and Singh, 1972). As those skeletal elements undergoing more rapid rates of growth are considered to be more sensitive to deleterious growth conditions (Bogin and Varela-Silva, 2010), this would result in the femur being more impacted by poor maternal health *in utero* than both the tibia and the humerus, aligning with the findings of the present study.

As the impoverished nature of the study sample is based on parental socioeconomic status (Belcastro et al., 2017), it stands to reason that the mothers of the observed individuals would have experienced negative biocultural factors associated with poverty, such as inadequate nutrition, poor sanitation, and disease. When these conditions are present during pregnancy, accompanying health issues and nutrient deficiency can lead to immature fetal development (Pozzi, 2000). Archival evidence from this period highlights the awful dietary conditions of the working class in the Bologna region. For example, historical accounts document their diet as being “terrible” and consisting mainly of grains with low nutritional value, primarily corn-based items such as polenta (Fornasin, 1998; Hogan and Kertzer, 1987; Livi Bacci, 1990). In addition to being comparatively lower in calories than other grains, diets high in corn are also associated with pellagra, a condition stemming from vitamin B3 (niacin) deficiency (Dalla-Zuanna and Rosina, 2011). Records show that the condition was prevalent in northern Italy in the late 19th century (Livi Bacci, 1986), with statistical records on population health from this time identifying a link between ill, undernourished parents and frail offspring (Sormanni, 1881). This relationship between maternal malnourishment and under-weight newborns has also been observed in modern developing countries (WHO, 1995).

The effects of this nutrient lacking diet would have been exacerbated by the unsanitary and poor living conditions of Bologna at this time. As a result of Italy's industrial revolution during the 19th century (Kertzer, 1987), the city saw a rapid increase in both its population and pollution, with many residents of its surrounding towns moving to the urban centre (Hogan and Kertzer, 1985). The combination of overcrowding and inadequate sanitation infrastructure led to frequent infections and chronic malnutrition among Bologna's lower socioeconomic classes. In particular, the consumption of unsanitary water from Bologna's well and canal systems has been identified as a cause of many health concerns. Favoured by the city's poorer citizens for the free water they provided, these water sources posed an extreme risk to the health of those who used them. Historical documents attribute recurrent and widespread cholera and typhoid epidemics in Bologna to the contaminated water supply (Drusiani et al., 2010; Faggioli, 2006; Hogan and Kerzter, 1987). These unhygienic conditions and accompanying infectious diseases would have taken a toll on the physical condition of Bologna's impoverished population, with death records for the Certosa collection indicating that approximately 30% of the individuals died as a result of infectious and parasitic diseases, including gastroenteritis (Belcastro et al., 2017).

These factors support the theory that the mothers of the Certosa children experienced poor health, both prior to and during their pregnancies, which in turn may have resulted in poor health and accompanying diminished growth of their offspring. Studies examining the relationship between socioeconomic status (SES) and birth weight have found that neonates from low socioeconomic groups have a greater frequency of low birth weight than those from higher social groups, indicating a link between maternal SES and fetal development (Scalone and Samoggia, 2018; Ward, 1993). Statistical studies of micro-data on socioeconomic conditions and high infant mortality rate in northeastern Italy from this time period support the causal effect that poor maternal health would have had on the physical condition of their infants (Dalla-Zuanna and Rosina, 2011; Scalone and Samoggia, 2018). Death records from the late 19th century cite "infantile atrophy" as the cause of death in approximately 60% of infants born in the region (Direzione Generale della Statistica, 1890). At this time, the condition was diagnosed in instances when an infant was underweight or

delayed in their physical development as a result of maternal malnourishment or congenital factors (Manfredini and Pozzi, 2004). In cases of poor maternal health, it is expected that fetal development would be impacted from early on in the *in utero* period. This would explain why even those bones which form relatively early, such as the femur, show diminished growth. Similar results have been found in research into the body proportions of full-term neonates determined to be small-for-gestation, where prolonged periods of intrauterine growth retardation have been linked with growth delays in earlier forming skeletal elements (Brooke, Wood, and Butters, 1984).

Further evidence in support of this explanation is found in the differential growth stunting observed between the males and females of the study sample. The Certosa males experienced more diminished growth than the females for all skeletal elements, although not significantly so. These findings are in line with existing literature, which has demonstrated that males are more susceptible to the adverse biocultural conditions found in deleterious growth environments (Stin, 1969; Stinson, 1985). One possible interpretation of this observation is that individuals within this population did not follow gender-biased child-rearing practices. In this situation, the greater sensitivity of males to growth perturbations is not mediated by preferential treatment over their female counterparts, therefore they continue to experience relatively more diminished growth as a result. An examination of data on mortality and disease rates by sex from this time period in Italy do not support this interpretation. Statistical records for northern Italy from the late 19th century indicate that although males did experience a greater risk of neonatal mortality than females (Hogan and Kertzer, 1987), the rates reach near equal levels within the first year of life (Manfredini, Breschi, and Fornasin, 2017). By the third year of life, risk of death is notably higher in girls (Manfredini, Breschi, and Fornasin, 2017), as are rates of disease (Pinnelli and Mancini, 1999). These data are suggestive of cultural practices which favoured males and put females at a disadvantage. In light of this, an alternative interpretation of the observed sex differentials in growth delays is that the disruption began prior to birth, as it would not be possible to act on gender biases during the fetal period while sex of the child was unknown. This would align with the greater growth deficit observed in the Certosa males, in spite of

evidence that they receive preferential treatment and a more favourable postnatal growth environment than the females. It is possible that the growth delays experienced *in utero* could not be mediated by the advantages they received after birth, which research shows can be difficult to recover from (Chung and Kuzawa, 2014; Dewey and Mayers, 2011).

This is particularly true for children subject to poor growth environments, such as those present in the study sample. It is very likely that the negative biocultural factors responsible for poor health of the mothers would have impacted the children of the Certosa collection postnatally as well. When an individual experiences long-term stress, such as chronic malnutrition or illness, throughout their developmental period, it impacts their ability to recover from delayed growth (Dewey and Mayers, 2011). Additionally, evidence shows that individuals whose growth is already stunted at the time of stress episodes are unlikely to undergo 'catch-up' growth following cessation of the stressor (Dewey and Mayers, 2011), thereby compounding the effects of the growth faltering and falling further off their expected growth curve. In these circumstances, the finding of the femur exhibiting the greatest level of growth stunting could be attributable to it undergoing the relatively greatest amount of growth, in addition to having an extended exposure to negative biocultural factors, during both the *in utero* and postnatal periods (Cunningham et al., 2021).

While the observation of greater stunting in the proximal segment of the lower limb does not align with the expected research outcomes, this study is not the first to find such a trend. In their analysis of childhood growth throughout periods of religious transitions in Portugal, Gooderham and colleagues (2019) found the femur to exhibit mean z-scores lower than those of the tibia, while Cardoso (2005) observed femoral growth delay which was equal or greater than that of the tibia when evaluating the growth of impoverished Portuguese children from the 20th century. Similar results were found by Temple and colleagues (2014) in their comparison of skeletal growth between temporally distinct groups of foragers in Eastern Siberia. Significantly greater levels of femoral stunting were detected in individuals from the earlier group, who were believed to have experienced increased systemic stress, while no significant difference was found in the tibial lengths of the two groups. An analysis

of growth in populations comprised of enslaved African children and impoverished black communities in 1920s America (Cardoso et al., 2018) revealed that two of the three samples had greater growth stunting in the femur than the tibia. The results found by these and other studies (Holliday and Ruff, 2001; Johnston, 1962; Pinhasi et al., 2006) indicate that our understanding of growth and varying sensitivity may require further consideration.

Beyond the archaeological research cited above, an examination of clinical literature reveals that femoral stunting is not an unusual occurrence in modern health settings, particularly during the prenatal stage. Because of the known association between short femur length and a wide range of adverse health outcomes, the femur is the only long bone required by international guidelines to be routinely measured at fetal ultrasound appointments during the second and third trimester (D'Ambrosio et al., 2019; Li et al., 2022). The measurement is considered to be an early indicator of placental insufficiency (Zalel et al., 2002), intrauterine growth restriction (Bromley et al., 1993; Goetzinger et al., 2012), and low birth weight (Friebe-Hoffman et al., 2022; Mailath-Pokorny et al., 2015), with approximately 20% of fetuses classified as having short femur length being born as small-for-gestational age (Mathiesen et al., 2014). Research shows that the postnatal development of children who are born small-for-gestational age has the potential to be impacted throughout their life, with approximately 10-30% of individuals not experiencing catch-up growth and achieving a stunted terminal stature (Albertsson-Wikland and Karlberg, 1997; Cianfarani, Ladaki, and Geremia, 2006; Leger et al., 1997). This suggests that those individuals presenting with reduced femoral lengths early in life may then also be subject to diminished growth overall, particularly in instances where their socioeconomic conditions are not conducive for catch-up growth, such as those experienced by the children of the Certosa collection.

2.6. Conclusion

This study explores the differential sensitivity of the limb dimensions within the theoretical framework of the *laws of developmental direction*. By using a documented skeletal collection of known socioeconomic status, it was possible to assess variability in diminished growth for

age in impoverished children. The findings of this research indicate that the children of the Certosa collection demonstrate delayed or diminished skeletal growth in comparison to the reference sample, however the results also show that the regions of the body are not equally affected by biocultural factors and do not align with the theorized developmental gradient. While the lower limb was found to be more delayed in growth relative to the upper limb, and the distal segment of the upper limb exhibited a greater magnitude of diminished growth than was observed in its proximal segment, comparison of the bones in the lower limb found the proximal bone to be significantly more delayed in growth in comparison to both bones of the distal segment. This is an unusual observation which brings into question the applicability of assumptions stemming from the *laws of developmental direction*. It is apparent from these results that the regions of the body are not equally affected by biocultural factors, with some bones showing markedly more diminished growth than others. It is believed that the varying timeline and tempo of growth observed in these bones are responsible for the differential growth patterns observed in the present study, which in turn may align with biocultural stressors present at different human life history stages. The findings of this research indicate that a greater understanding of early life experiences, both at the individual and maternal level, may be gained through the comparative study of diaphyseal growth in both the proximal and distal limbs and their segments.

3. Do the regions of the spinal column record stress differently? An analysis of diminished growth in the vertebral neural canal

3.1. Abstract

OBJECTIVE: The diameter of the vertebral neural canal (VNC) is commonly used as an indicator of non-specific stress occurring early in life. The majority of studies which assess the VNC prioritize measurements of the lumbar region, as these vertebrae are considered by some to have the greatest potential for recording growth disruption due to a longer postnatal growth period. This study explores the validity of such a focus by comparing cervical, thoracic, and lumbar VNC diameters of 29 children (3-18 years) from the documented Certosa skeletal assemblage.

METHODS: Published vertebral diameters were used to generate z-scores for anterior-posterior diameters for the cervical and lumbar vertebrae and for transverse VNC diameters for all vertebral sections. Diminished growth was defined as ≤ -2 standard deviations (SD). Repeated Measures ANOVA was used to assess differences in mean APD and TRD z-scores for each vertebral region.

RESULTS: Growth reduction in the transverse diameter of the cervical region was significantly greater than in the lumbar ($p \leq 0.001$) and thoracic regions ($p \leq 0.001$), which were not significantly different from one another ($p = 0.524$). Repeated Measures ANOVA indicate that while the lumbar vertebrae have lower mean values than the cervical vertebrae, these differences are not significant ($F[1,20] = 2.259$, $p=0.148$).

CONCLUSION: Contrary to expectations, the TRD of the cervical vertebrae was found to be more diminished in growth than both the thoracic and lumbar regions, which does not align with current methodological practices that exclude cervical data from analyses. Additionally, comparative analysis of the APD indicate that the cervical vertebrae may be similarly useful at detecting evidence of early life stress relative to the lumbar vertebrae. These findings indicate that a focus on the lumbar region alone could overlook growth insults recorded by the earlier developing cervical vertebrae.

3.2. Introduction

The dimensions of the vertebral neural canal (VNC) are commonly used by biological anthropologists as a non-specific indicator of stress when studying physical remains in archaeological contexts. These measurements, along with other skeletal and dental markers, aid in reconstructing the lived experiences of individuals from past populations and provide a record of their social and environmental conditions. The timing of neuro-osseous tissue development causes the diameters of the VNC to be uniquely suited for recording episodes of stress occurring in the first years of life (Clark et al., 1986). Development of the neural canal diameters begins during the second month *in utero*, with completion timelines varying by both region and diameter (Louryan et al., 2011; Cunningham et al., 2017). Compared to other skeletal and dental elements, the bones of the vertebrae undergo relatively rapid growth that occurs in the first years of life (Clark et al., 1986; Hinck et al., 1966; Cunningham et al., 2017), with the diameters of the neural canal attaining approximately 95% of their final size by five years of age (Dimeglio, 1992). This results in vertebral elements being highly sensitive to growth disruptions stemming from biocultural factors, such as disease, malnutrition, and adverse environmental conditions during this early stage of growth (Brooke et al., 1984; Papp et al., 1994; Platt & Stewart, 1962). The early fusion of the vertebral elements limits the potential for catch-up growth, effectively locking in or preserving evidence of stunted growth (Tanner, 1978; Hinck et al., 1962). As such, morphometry of the neural canal in older children and adult individuals can provide the opportunity to observe effects of in utero and early childhood stress many years after their occurrence (Clark et al., 1986).

Although commonly used in the analysis of past populations, many studies (Corron et al., 2021, 2023; Papp et al., 1994; Porter & Pavitt, 1987; Rewekant, 2001; Watts, 2011, 2013a, 2013b, 2015) which assess reduced dimensions of the VNC prioritize measurements of the lumbar region over vertebrae in the upper regions of the spine. The reasoning behind this focus is largely attributed to the longer growth period of the lumbar spine versus the cervical and thoracic regions. With the longest period of postnatal development, it has been

suggested that the lumbar vertebrae consequently have the greatest potential to record evidence of growth disruption (Clark et al., 1985; Watts, 2011). This focus on the lumbar spine is mirrored in clinical research into reduced VNC diameters (Aly & Amin, 2013; Amonoo-Kuofi, 1982, 1985; Griffith et al., 2016; Muthuuri 2021; Oguz et al., 2002; Pierro et al., 2017; Porter et al., 1987b; Ranjan Das et al., 2018; Tacar et al., 2003; van Roy et al., 2001; Verbiest, 1980), referred to as spinal stenosis, as the lumbar vertebrae are cited as the region most affected by the condition (Saldua et al., 2015; Waldman, 2022). An associated complication is that this narrow clinical focus has limited the available comparative metric data for VNC dimensions of the cervical and thoracic vertebrae (Hinck et al., 1962). It is possible that the resulting lack of reference standards has contributed to preferential examination of the lower spine when assessing VNC diameters in archaeological contexts.

While a longer lumbar developmental window may allow for growth insults occurring across a broader period of childhood to affect VNC development, it in turn would provide the lumbar vertebrae with the greatest potential for catch-up growth to occur when compared to the other two vertebral regions. Additionally, the shorter developmental timelines of the cervical and thoracic vertebrae mean that they undergo growth at a more rapid rate than the lumbar vertebrae (Baker et al., 2005; Clark et al., 1985; Clark et al., 1986). This results in their growth being comparatively more vulnerable to the effects of disruption, as a greater percentage of their overall development is impacted (Bass et al., 1999). Furthermore, the shorter developmental periods of the upper spinal regions offer less opportunity for catch-up growth to occur than in the lumbar vertebrae. It is therefore possible that the cervical and thoracic vertebrae have the potential to record earlier occurring or less severe episodes of stress than lumbar vertebrae, unaltered by catch-up growth. If this is the case, the practice of limiting archaeological data collection and analysis to the vertebrae of the lumbar region may be missing valuable and informative skeletal evidence located in the VNC diameters of the upper regions of the spine.

The intent of this study is to compare diminished growth in the cervical, thoracic, and lumbar neural canals and determine how the differing developmental timelines and tempos of the

three vertebral regions might impact their ability to record the effects of adverse biocultural environments. It is intended that this research will lead to more informed methodological approaches to VNC data collection and increasingly thorough analyses of disrupted growth in past populations.

3.3. Materials and Methods

The study sample comes from the Certosa collection, a well-documented skeletal assemblage comprising the remains of 425 individuals from 19th century Bologna, Italy (Belcastro et al., 2017). Exhumed from the Certosa Cemetery in the early 1900s, these individuals were located in those areas of the cemetery designated for the city's lower classes (Vidor, 2012). Associated records for the adults within the collection provide further evidence of the impoverished status of these individuals, with the majority of men employed as unskilled labourers, and the occupation of most women listed as housewife (Belcastro et al. 2017). Previous research (Cardoso et al., 2018; Nelson et al., 2017, 2021, 2023) into the skeletal and dental development of the Certosa children has found that factors related to their socioeconomic conditions resulted in severely delayed or compromised growth and development.

In total, the vertebrae of 29 juvenile individuals (n = 14 females, n = 15 males) were analyzed for this study (Table 3.1). As fusion of the neurocentral synchondrosis does not occur until approximately 3 years of age (Cunningham et al., 2017), the study sample consists of children ranging in age from 3-18 years at the time of their death. Unlike similar studies into the vertebral development of archaeological samples (Clark et al., 1986; LoPresto, 2020; Newman & Gowland, 2015; Watts, 2013), the sex and age at death of individuals within the study sample is known from associated birth and death records. This allows for a unique level of certainty not typically possible in archaeological contexts, where age is frequently estimated based on skeletal and dental maturation, which makes assessments of development-for-age difficult. While it would be possible to assess for differences in canal

growth between the sexes in the current study, the sexes were pooled due to small sample size.

To facilitate accurate z-score comparisons with available reference standards (Hinck et al., 1962; Hinck et al., 1965; Hinck et al., 1966), individuals were grouped into the following age categories: 3.0-5.9 years, 6.0-8.9 years, 9.0-10.9 years, 11.0-12.9 years, 13.0-14.9 years, 15.0-16.9 years, and 17.0-18.9 years. These age categories were chosen to best align with the reference data, as well as existing research involving VNC analysis (Watts, 2013a, 2013b, 2015) and the Certosa collection (Nelson et al., 2021).

TABLE 3.1. Age and sex composition of the study sample.

Age Group (years)	Females (n)	Males (n)	Total (n)
3.0-5.9	5	7	12
6.0-8.9	3	1	4
9.0-10.9	1	1	2
11.0-12.9	0	2	2
13.0-14.9	0	1	1
15.0-16.9	2	2	4
17.0-18.9	3	1	4
Total	14	15	29

The study sample included all present and undamaged vertebrae where fusion had occurred in the appropriate plane. As age of fusion differs for the anterior-posterior diameter (APD) and transverse diameter (TRD) (Cunningham et al., 2017), sample sizes for each diameter will vary. Any vertebrae where trauma or pathological condition were observed were excluded. Direct measurements of the vertebral neural canal were taken using Mitutoyo callipers (Absolute Digimatic, Series 572) and recorded to the nearest hundredth of a millimetre. APD of the VNC was recorded as the maximum distance from the most anterior part of the neural arch to the posterior surface of the vertebral body, while the TRD was recorded as the maximum distance between the medial surfaces of the left and right pedicles. Due to morphological differences in VNC of the first and second cervical vertebrae, these were excluded from the study sample. A total of 576 vertebrae were measured, resulting in 183

measurements of the APD and 575 measurements of the TRD. For a summary of all sample measurements and descriptive statistics, see Appendices C and D.

Measurement error was tested by comparing measurements taken on two occasions, four weeks apart. A sample of 20 vertebrae was measured in the APD and the TRD by one observer, and then re-measured by the first observer and a second observer. The relative technical error of measurement (%TEM) and coefficient of reliability (R) were calculated following the method outlined by Ulijaszek and Kerr (1999). Intra- and inter-observer error was assessed by comparing observations through a single calculation of %TEM and R. The results of these calculations for both the transverse and anterior-posterior diameters (R=0.99) indicate that less than 1% of the variance between intra- and inter-observer measurements is due to factors related to observer error. Based on this, the measurements used in this study can be considered reliable.

Standard, or z-scores were calculated to determine if VNC diameters in the study sample were diminished compared to the mean dimensions from reference samples. The following formula was used for these calculations: $z = (x - \mu) / \sigma$ where x is the VNC diameter of each vertebra from the Certosa sample, μ is the vertebra-specific mean VNC diameter from the reference sample, and σ is the vertebra -specific standard deviation of the reference sample. z-scores were calculated for the APD of cervical vertebrae 3 through 5 using Hinck et al. (1962) and for all lumbar vertebrae using Hinck et al. (1965). z-scores for the APD of thoracic vertebrae could not be calculated due to a lack of appropriate reference data. Hinck et al. (1966) provided the reference data for the TRD for all three vertebral sections. The reference data from Hinck and colleagues were measured from roentgenograms of living children and may be impacted by the magnification which is incorporated into radiographic images. As the distance from subject to film was not provided in these publications, no correction of these data was possible. The impacts of this magnification on the study results are discussed below. As the age and sex is known for all individuals within the study sample, sex and age specific reference data were used when calculating z-scores.

The mean z-score for each vertebral region was determined in order to identify those individuals who experienced diminished VNC growth. Individuals were identified as having diminished growth if they had an average z-score two SD below the reference sample mean of one or more vertebral regions, based on existing definitions of growth stunting (WHO Expert Committee on Physical Status, 1995). Repeated Measures ANOVA with age as a covariate and a Bonferroni correction was used to assess differences in z-score means of the TRD and APD between each vertebral region.

3.4. Results

Of the 29 individuals in the study sample, 26 (90%) produced a mean z-score of ≤-2 in one of the VNC dimensions in at least one vertebral region. As the transverse and anterior-posterior diameters develop on differing timelines (Clark et al., 1985; Cunningham et al., 2017), the results of the statistical analyses are examined separately.

3.4.1. Transverse Diameters

Of the 29 individuals with TRD data, 76% of individuals (n=22) were found to have a mean z-score of ≤-2 in the TRD in at least one vertebral region. Broken down by region, 69% (n=20) had a mean z-score of ≤-2 in the TRD of the cervical region, 17% (n=5) in the thoracic region, and 31% (n=9) in the lumbar region. The mean z-scores for the transverse diameter measurements for the cervical, thoracic, and lumbar vertebral regions are shown in Table 3.2. z-score means ranged from -2.93 to -0.96. The cervical region had the lowest mean z-score across all age groups and averaged values below -2 in four of the seven age groups. The thoracic region had a mean z-score value below -2 in only one age group (9-10 years), while the mean z-scores of the lumbar region did not produce a z-score mean below -2 in any age group. z-score means are lowest for the cervical and thoracic vertebral regions in the 6-8 (cervical, z = -2.93; thoracic, z = -1.75) and 9-10 year (cervical, z = -2.67; thoracic, z = -2.01) age groups, with the lumbar region showing the lowest z-score mean values in the 3-5 (z = -1.85) and 9-10 (z = -1.97) year age groups.

TABLE 3.2. Mean transverse diameter (TRD) z-score values by vertebral region and age category.

	Cervical			Thoracic			Lumbar		
Age Group (years)	*n*	Mean	*SD*	*n*	Mean	*SD*	*n*	Mean	*SD*
3.0-5.9	37 (10)	-2.02	0.99	125 (11)	-1.22	0.85	49 (12)	-1.85	0.72
6.0-8.9	16 (4)	-2.93	0.74	46 (4)	-1.75	0.91	20 (4)	-1.14	0.66
9.0-10.9	8 (2)	-2.67	0.59	23 (2)	-2.01	1.14	10 (2)	-1.97	0.32
11.0-12.9	10 (2)	-1.67	0.49	24 (2)	-1.44	0.81	10 (2)	-1.28	0.66
13.0-14.9	3 (1)	-1.63	0.63	12 (1)	-1.09	0.36	5 (1)	-0.96	0.19
15.0-16.9	20 (4)	-2.49	0.90	48 (4)	-1.24	1.19	20 (4)	-1.64	1.02
17.0-18.9	17 (5)	-1.70	0.56	58 (5)	-1.39	0.78	18 (5)	-1.04	0.47
Total	111 (27)	-2.19	0.86	324 (28)	-1.39	0.96	132 (29)	-1.54	0.75

n, number of vertebrae (number of individuals); *SD*; standard deviation.

Inter-vertebral comparisons were possible in 27 individuals, where dimensions for all three vertebral regions were measurable. Out of this group, four individuals averaged a z-score of <-2 in every region (mean z-score values: cervical = -3.37, thoracic = -2.65, and lumbar = -2.48), while eight have z-score values of <-2 in at least two vertebral regions. The box plot in Figure 3.1 demonstrates the differences in mean z-score distributions between the three vertebral regions. These box plots highlight that the difference in mean z-scores is found consistently in the sample, with the cervical region visibly lower than the other two in both range and median values. Differences in mean TRD z-scores were found to be statistically significant through Repeated Measures ANOVA ($F[2,50] = 7.29$, $p = 0.002$) with age as a covariate. Results indicate that age had no significant effect on the level of diminished growth ($F[2, 50] = 1.60$, $p = 0.213$). Post-hoc tests indicate that cervical vertebrae differed significantly in mean z-score values from both thoracic ($p \leq 0.001$) and lumbar vertebrae ($p \leq 0.001$), while no significant difference was found between these two regions ($p = 0.524$).

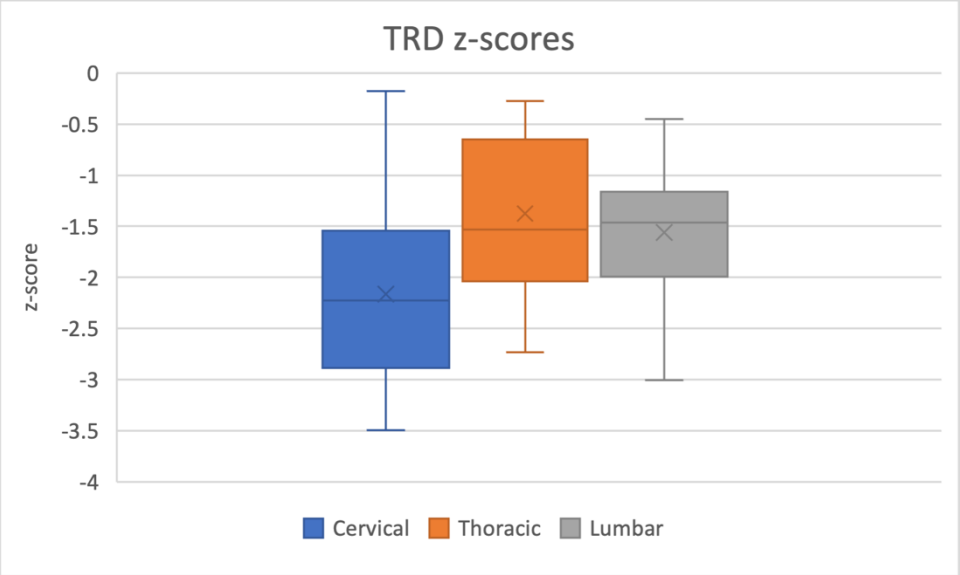

FIGURE 3.1. Box plots of mean TRD z-scores for the cervical, thoracic, and lumbar vertebral regions comparing the distribution between each. The line in the box represents the median score and the lower and upper ends are the 25th and 75th quartiles. The x in each box represents the mean z-score.

3.4.2. Anterior-Posterior Diameters

Of the 29 individuals with APD data for cervical vertebrae 3-5 and lumbar vertebrae 1-5, 23 (77%) have a mean z-score of <-2 in at least one vertebral region. Out of 22 individuals with cervical vertebrae APD measurements, 45% (n=10) have a mean z-score of <-2. Of 29 individuals with an AP diameter for lumbar vertebrae, 66% (n=19) have a z-score of <-2. Mean z-score values for the AP dimension ranged from -3.60 to -1.06 (Table 3.3). The lumbar region was <-2 in all but one age group (9-10 years) and had the lowest mean z-score in all age groups, with the exception of one (17-18 years). z-score means are lowest for the cervical region in the 17-18 year group, while the lumbar region shows the lowest z-score mean values in the 13-14 year age group.

TABLE 3.3. Mean anterior-posterior diameter (APD) Z-score values by vertebral region and age category.

	Cervical			Lumbar		
Age Group (years)	*n*	Mean	*SD*	*n*	Mean	*SD*
3.0-5.9	22 (9)	-1.79	0.91	56 (12)	-2.22	1.03
6.0-8.9	6 (2)	-2.05	0.41	17 (4)	-2.73	0.94
9.0-10.9	3 (1)	-1.06	0.28	5 (2)	-1.73	1.25
11.0-12.9	6 (1)	-1.82	0.71	10 (2)	-2.10	1.55
13.0-14.9	3 (1)	-1.59	0.05	5 (1)	-3.60	1.99
15.0-16.9	11 (4)	-1.58	0.56	20 (4)	-2.34	1.37
17.0-18.9	8 (5)	-2.76	1.02	18 (5)	-2.24	0.97
Total	55 (22)	-1.84	0.57	128 (30)	-2.46	0.73

n, number of vertebrae (number of individuals); *SD*; standard deviation.

There are 22 individuals that have AP dimensions for both cervical and lumbar vertebrae. Of these 22, six have z-score means <-2 in both the cervical and lumbar regions, with mean z-scores of -2.44 and -2.61 respectively; nine have z-score means <-2 only in the lumbar vertebrae (-2.66) and 4 have z-score means <-2 in just the cervical vertebrae (-2.65). The box plot in Figure 3.2 demonstrates the differences in mean z-score distributions between the cervical and lumbar vertebral regions. Repeated Measures ANOVA with age as a covariate found that the differences in mean APD z-scores of the cervical and lumbar were not statistically significant ($F[1,20] = 2.259$, $p=0.148$). Results indicate that age had no significant effect on the level of diminished growth ($F[1, 20] = 0.218$, $p = 0.646$).

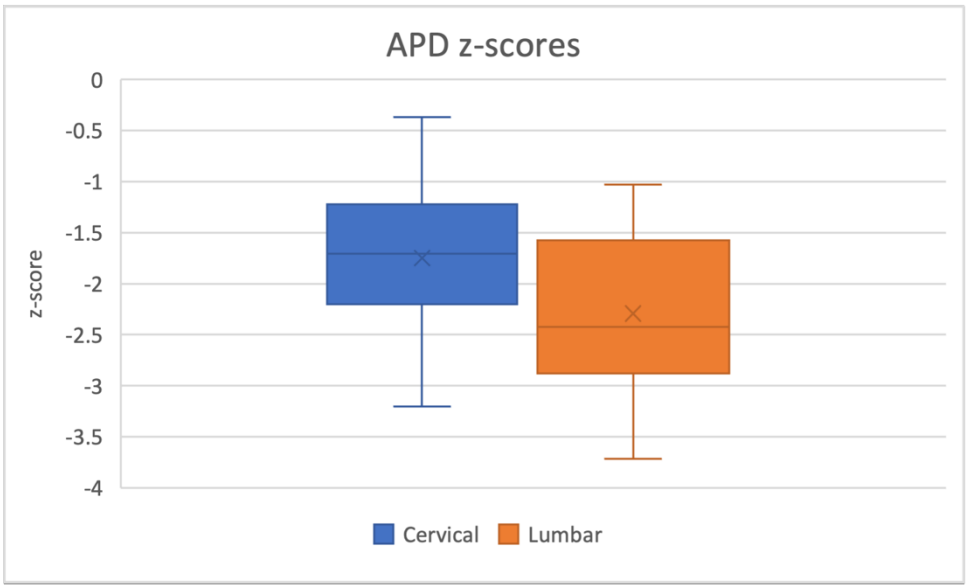

FIGURE 3.2. Box plots of mean APD z-scores for the cervical and lumbar vertebral regions comparing the distribution between each. The line in the box represents the median score and the lower and upper ends are the 25th and 75th quartiles. The x in each box represents the mean z-score.

3.4.3. Transverse and Anterior-Posterior Diameters

In total, 20 individuals (69%) were found to have z-score means <-2 in both the transverse and anterior-posterior dimensions of the spinal canal. Of these individuals, 14 have z-score means <-2 in both diameters of the same vertebral region (n=5 cervical, n=9 lumbar), while 16 individuals produce z-score means <-2 APD & TRD growth of different vertebral regions.

3.5. Discussion

The results of this study provide evidence of differential growth disruption in the vertebral neural canal between the regions of the spine. Specifically, the TRD of the cervical vertebrae was found to be significantly more diminished in growth than either the thoracic or lumbar regions, while these two areas did not differ significantly from each other. Additionally, although the APD z-score means for the lumbar vertebrae were lower than those of the

cervical vertebrae, this difference was not statistically significant. These are notable findings as the vast majority of studies which assess growth of the VNC do not include cervical vertebrae in their analyses, with only two studies (Amoroso & Garcia, 2018; Newman & Gowland, 2015) being identified at the time of writing which examined the diameters of all vertebral regions. These results demonstrate that the preferential examination of the lumbar vertebrae in studies exploring early life stress may be overlooking growth outcome information captured by the upper regions of the spine. This is particularly important as the differing developmental timelines of the vertebral regions hold the potential to inform researchers on biocultural insults occurring at various points during in utero development and throughout the first years of childhood, thereby increasing our understanding of the lived experiences in a population.

There are several possible explanations for these varying levels of growth disruption, one of which pertains to issues associated with the comparative reference data. As previously mentioned, the TRD data from Hinck et al. (1966) was not corrected for radiographic magnification bias, which may impact results when directly comparing measurements taken from dry bone. Although all radiographic images for the TRD were taken from the same distance (40 inches), there may be some variation in the magnification factor within the spinal column due to its natural curvature, which results in some vertebrae being positioned farther from the film than others. This would have the greatest impact in the thoracic region, specifically for those vertebrae involved in the kurtotic curve, as they would be closer to the film and therefore subject to less magnification relative to the cervical and lumbar vertebrae. This may be a contributing factor to the lack of diminished growth found in the thoracic vertebrae. The authors do not report the distance from subject to film, making correction of the data impossible. These magnification issues may also apply to the study findings for the APD. Neither of the studies used for the cervical (Hinck et al., 1962) and lumbar (Hinck et al., 1965) reference data corrected their APD measurements for magnification, resulting in potential issues for comparison of measurements from dry bone. Unlike with the TRD data, the radiographic images used in the two APD studies were taken from different source to subject distances, with the source positioned 60 inches from the

cervical vertebrae and 40 inches for the lumbar vertebrae. Unfortunately, the magnification factor for these data cannot be calculated at present as the distance of the source to the film has not been published, however in their 1965 article, the authors estimated radiographic magnification to be 3mm for the lumbar images (Hinck et al., 1965). A 2008 study from Ravi & Rampersaud found that when captured from the same distance, lateral radiographs of lumbar vertebrae had a magnification error of approximately 30%, while the error for cervical vertebrae was approximately 20%. This indicates that the lumbar APD values reported by Hinck et al. (1965) may be 10% more magnified than the cervical APD values (Hinck et al., 1962). In addition, radiographic images taken at a closer source to subject distance have a higher magnification factor than those taken from farther away (Cesar et al., 2020). As the lumbar radiographs were taken at a closer source to subject distance than the cervical images, this may also indicate a higher magnification error in the reported lumbar APD values compared with the cervical APD values. The true effect of the magnification biases on the results of this study cannot be determined, however it is possible given the cited information that the Certosa children may have experienced greater levels of diminished APD growth in their cervical vertebrae than are reported in this study. Future research involving VNC diameters as a biomarker in past populations would greatly benefit from the creation of more complete reference datasets representing normal vertebral growth, specifically those with corrected measurement values or using imaging technology which is free from magnification bias, such as CT.

A further consideration relevant to the discussion of these research findings is the potential impacts of the osteological paradox on this study. When comparing the growth of children whose lives were ended prematurely due to their biocultural conditions with the growth of living children, there are biases present which need to be acknowledged. In addition to adversely affecting the growth of their vertebral elements, the negative developmental factors experienced by the Certosa children also increased the likelihood of them dying. Research has demonstrated a higher risk of mortality in children with diminished growth than is observed in children experiencing normal development (Martorell, 1989; Pelletier et al., 1994). This in turn makes it more likely that diminished growth will be observed in the

study sample, while also potentially resulting in differing growth patterns than would be found in a living population, such as those from which the comparative VNC data were measured. The effects of this biological mortality bias (Wood et al., 1992; Saunders & Hoppa, 1993) should be considered when exploring potential interpretations or patterns of diminished VNC growth in the Certosa collection.

Potential impacts of the mortality bias on study findings are also apparent when examining the Certosa collection's mortality profile, which indicates that approximately 48% of individuals in the juvenile segment of the assemblage died before their first birthday (Belcastro et al., 2017). This figure is in line with the infant mortality rate for the rest of Italy, which at this time had one of the highest amongst European countries (Kertzer et al., 1999; Scalone et al., 2016). As stressors which impact growth are generally chronic in nature (Selye, 1978; Vercellotti, 2011), individuals who are unable to survive more acute adverse biocultural conditions are unlikely to have their skeletal growth impacted. This phenomenon has been identified in previous research (Wood et al., 1992), where frequency of diminished growth is low in periods of high mortality. Although the mortality rate for the Certosa collection is high in the first year of life, there are relatively low levels of diminished growth observed in vertebral dimensions completing their growth during this time, such as the TRD of the thoracic vertebrae which undergoes development prior to one year of age (Cunningham et al., 2017). This suggests that while acute stressors were frequent among the infants of the Certosa collection, chronic stress was not commonly experienced within the first year of life. Exposure to these acute types of stressors would not only result in death occurring before skeletal growth may be affected, but also prior to fusion of the vertebral elements. As the study sample only includes individuals over the age of three, who have completed fusion, this would result in these children not being included in the sample, thereby preventing analysis of their canal growth.

This sample criterion may itself have an impact on the research outcomes of this study. As accurate dimensions of the neural canal cannot be measured from dry bone prior to epiphyseal union between the relevant vertebral elements, the study sample includes only

individuals where this has occurred. Research shows that although the process of epiphyseal fusion is relatively more robust to the impacts of deleterious biocultural factors than skeletal growth, it is not wholly immune, with delays in maturation observed in several studies (Cardoso; 2008a; 2008b; Frisancho et al., 1970; Lewis et al., 2016; Shapland & Lewis, 2013; Temple et al., 2021). Individuals from low socioeconomic backgrounds or impoverished environments have been found to be delayed in the age at which epiphyseal fusion occurs when compared with individuals of higher socioeconomic status (Frisancho et al., 1970; Lewis et al., 2016). For the present research, this would mean that young individuals experiencing poor growth conditions may be excluded from the study sample as a result of delayed fusion between the vertebral elements. The impact that these delays in maturation have on age of fusion specifically between spinal epiphyses has been largely unexplored until recently. A 2021 study from Temple and colleagues examined various fusion points to assess for variation in maturation delays of skeletal elements in a hunter-gatherer population known to have experienced physiological stress. Their research found that the observed age of fusion for the vertebral elements was in line with expectations from reference standards, although the cervical and thoracic neural arches did exhibit some delay (Temple et al., 2021). This may be a contributing factor to the low levels of diminished growth found in the TRD of thoracic region in the present study. The impact of biocultural stressors on vertebral maturation is an area of research which would benefit from additional research.

Also of interest is the level of post-fusion "growth" that has been noted to occur in the VNC. While the dimensions of the neural canal are not considered to increase in size in the APD once fusion has occurred (Watts, 2013b), increases in the TRD have been documented in cross-sectional datasets up until the age of 15-17 years (Hinck et al., 1966; Newman and Gowland, 2015; Watts, 2013b). The extent to which the TRD increases in size beyond the point of fusion varies between studies, with research indicating that approximately 83%-95% of final size has been achieved by the age of fusion (3 years) (Dimeglio, 1992; Hinck et al., 1966; Newman and Gowland, 2015; Watts, 2013b). This increase in TRD has been interpreted in some studies as evidence that analysis of this osteometric data can provide information on an individual's growth and lived experiences during later childhood and

adolescence. There are several issues with these types of interpretations, the first being the nature of the study samples in which these increases have been observed. Several of the foundational bioarchaeological studies which document increasing TRD were conducted using skeletal remains from archaeological populations (Newman and Gowland, 2015; Papp et al., 1994; Watts, 2013b). In light of this, these results may have biases present which stem from the osteological paradox. The identified increases in size with age in the TRD may be due to differential growth occurring in individuals within the older age categories, whose biological circumstances were not as severe as those experienced by those individuals who died prematurely and therefore make up the younger age categories. This may then explain why those individuals who did not survive childhood have smaller TRD than those who did, resulting in observations of increasing TRD size with age. Furthermore, the cross-sectional nature of the data used in these studies raises additional concerns when making conclusions of growth occurring after fusion, as these inferences are based on measurements of different individuals and are therefore an approximation of a growth trajectory, rather than direct evidence of an individual's growth.

Another issue with interpretations of growth and lived experiences based on increasing TRD after the age of fusion is whether this increase in size can be attributed to "growth" as the term is used in developmental processes. Following fusion, changes in the neural canal are the result of bone remodeling, which may lead to alterations in the overall shape of the canal (Cunningham et al., 2017). The impact that biocultural stressors, such as illness and malnutrition, have on this remodeling process is unclear, with existing research (Stephensen, 1999; Suarez-Bregua et al., 2018; Tsukasaki & Takayanagi, 2019) showing that the activity of the osteogenic cells responsible for resorption and deposition of bone may be inhibited or promoted, depending on the type of stressor experienced. Additional research is needed to determine how this may impact age-related remodeling of the neural canal. The neural canals of the cervical and thoracic vertebrae do not show substantial changes to their shapes after fusion has occurred, while the process is quite pronounced in the lumbar vertebrae (Dimeglio, 1992; Tulsi, 1971; White et al., 2011). In this region, the canal shape changes from one that is ovoid to a more triangular or trifold canal shape, with the widest border following

the transverse axis (Reichmann and Lewin, 1971; Porter and Pavitt, 1987a; White et al., 2011). These changes in shape result in an increase in the TRD that is due to resorption of bone via remodeling, rather than being caused by additional growth of the vertebral elements (Reichman and Lewin, 1971). In this regard, increases in neural canal diameters after fusion are not a result of bone growth at all, therefore interpretations about an individual's growth environment and lived experiences based on post-fusion increases in TRD size warrant consideration. From a developmental perspective, a more apt term for this phenomenon may then be post-fusion "changes" rather than growth. For studies which use VNC diameters as a biomarker of early life experiences, the impact these post-fusion changes may have on their interpretations of diminished growth is largely dependent on the type of comparison occurring. Specifically, those studies which are evaluating the TR diameter of survivors with that of non-survivors, or the adults in a population with individuals who died during childhood, must consider the impact that these post-fusion changes may have on their research findings. As the present research uses age-specific reference data, where individuals are comparatively evaluated against those of a similar age, it is unlikely that changes due to post-fusion remodeling would alter the study outcomes.

The differing levels of reduced VNC growth observed between the segments of the vertebral column may be related to variation in the timing of their development and the varying biocultural factors occurring during these periods. While existing research generally agrees that approximately 70% of canal growth occurs in utero for all vertebrae (Clark, 1985; Dimeglio, 1992; Hinck et al., 1966; Watts, 2011), the ages at which the spinal regions complete the remainder of their development is quite variable. In the cervical region, the TRD of the neural canal undergoes its development prior to 2-3 years of age, when fusion of the posterior synchondrosis occurs (Cunningham et al., 2017). Comparatively, the neural arches of the thoracic vertebrae fuse between 1-2 years of age, meaning that the majority of postnatal TRD growth occurs within the first year of life (Cunningham et al., 2017). These timelines indicate that the cervical TRD is subject to a longer growth period than the thoracic vertebrae, resulting in a greater window of time during which potential growth stress may

occur as a result of deleterious biocultural factors, which could explain why the cervical region was found to have greater diminished growth relative to the thoracic region.

When considering the varying reduction in growth found between the cervical and lumbar TRD, this too is likely reflective of different biocultural factors associated with the varied developmental timelines of these vertebral regions. The lumbar TRD does not complete development until 2-3 years after that of the cervical vertebrae, with complete posterior synchondrosis fusion not occurring until approximately 5 years of age (Cunningham et al., 2017; Ursu et al., 1996). It is this lengthy window of development which has led to the near exclusive use of lumbar VNC measurements for the study of early life experiences in past populations. As was touched on previously, a longer growth period may equate to a greater window of time during which an individual might be exposed to growth disrupting biocultural factors. However, this extended developmental timeline also increases the potential for catch-up growth to occur in the TRD of the lumbar vertebrae (Clark et al., 1988; Jeffrey et al., 2003). Research has shown that in order for complete catch-up growth to occur in neuro-osseous tissue, such as the vertebral elements, cessation of the disruption must occur by approximately two years of age (Brandt, 1978; Clark, 1985; Scammon, 1923). As the development of the vertebral canals follows that of the neural growth curve (Brandt, 1978; Clark, 1985), this supports the possibility of catch-up growth occurring in these elements. However, with fusion of the posterior synchondrosis occurring between 2-3 years of age in the cervical vertebrae (Cunningham et al., 2017), it is unlikely that catch-up growth is possible in this region based on the aforementioned timeline. This is supported by findings from existing research comparing reduced VNC diameters of thoracic and lumbar vertebrae and age at death (Clark et al., 1986; 1988). Results indicate that later forming vertebral elements, such as lumbar TRD, have a weaker association with early death than those whose development is completed earlier, such as thoracic APD (Clark et al., 1986; 1988), which is interpreted as evidence of greater catch-up growth occurring in those elements forming later. It is posited that while the longer developmental period of the lumbar TRD allows for correction of deleterious growth conditions during the first years of life through catch-up growth, the earlier fusion of the cervical TRD prevents this from occurring.

Variation also exists in the ontogenetic timelines of VNC diameters within vertebral segments. The unique developmental periods of the TRD and APD of the neural canal means that their analysis provides evidence for lived experiences from different periods of early life. Although several studies have attributed the increased sensitivity of the lumbar vertebrae to their lengthier developmental timeline (Clark et al., 1985; Watts, 2011), it is not specified in these sources if this applies to the lumbar region in general or to a specific neural canal diameter. While the TRD of the lumbar vertebrae do experience the longest growth window relative to TRD in the rest of the spine, the APD of this region is shorter relative to that of the cervical vertebrae (Cunningham et al. 2017). The cervical vertebral elements are the first to begin forming in utero, with their neural arches appearing at approximately the sixth fetal week (Cunningham et al. 2017). Their APD growth continues until fusion at the neurocentral junction occurs, between 3-4 years of age (Papp et al., 1994). In comparison, the neural arches of the lumbar vertebrae begin ossifying around 3-4 months in utero and complete their APD development when fusion occurs between 2-4 years (Cunningham et al. 2017). Based on these ontogenetic timelines, the cervical and the lumbar vertebrae are exposed to the same biocultural factors during ontogeny of their APD. Although the differences observed between the APD of the two vertebral regions were not found to be significant, the smaller developmental window of the lumbar APD may contribute to the marginally greater diminished growth found in this dimension (Cunningham et al., 2017). With a shorter period of development, the lumbar APD would have less opportunity to undergo catch-up growth prior to fusion than the later fusing APD of the cervical vertebrae (Clark et al., 1986).

The shorter developmental timelines of the cervical TRD and lumbar APD not only result in less potential for catch-up growth, but also impact the likelihood of growth disruption through increased rates of growth in these diameters. Those skeletal elements experiencing faster growth rates are considered to be more sensitive to environmental perturbations than elements with a slower rate of growth (Baker et al., 2005; Bogin, 1999; Brandt, 1982; Eveleth and Tanner, 1990). This has been attributed to the fact that a relatively greater percentage of their growth is disturbed by episodes of stress (Baker et al., 2005; Brandt, 1982), thereby making it more difficult to overcome the adverse effects of stress exposures which occur

within the growth window. As the diameters of the neural canal are considered to be 70% complete at the time of birth (Clark, 1985; Dimeglio, 1992; Hinck et al., 1966; Watts, 2011), those vertebral dimensions with earlier fusion ages, such as the TRD of the cervical region and the APD of the lumbar region, must complete more of their growth within a shorter period of time. The results of this study demonstrate a greater sensitivity in the APD of the lumbar vertebrae which is not seen in the TRD. Notably, this dimension has the shorter of the two growth windows, suggesting that the faster rate of growth and reduced potential of catch-up growth of the APD lumbar vertebrae may be the cause of their sensitivity to growth disruption.

The patterning of growth reduction found in the VNC of the Certosa children indicate that they experienced stress before 2-3 years of age, with 45% of individuals exhibiting markedly diminished growth in the TRD of the cervical vertebrae and the APD of the lumbar vertebrae. Both of these dimensions complete their development on similar schedules, fusing between the ages of 2-4 years of age (Cunningham et al. 2017). One such stressor likely to have occurred during this period of development is weaning. Although there is documentation that employment within factories necessitated some Italian women to wean their infants quite early (Hogan and Kertzer, 1987), the majority of mothers for the children included in the present study were unemployed or had their listed occupation as “housewife” (Belcastro et al., 2017), which likely meant they were able to continue breastfeeding their infants for an extended period. Historical records indicate that this typically occurred around 10-12 months after birth in Northern Italian populations from this time period (Breschi and Livi Bacci, 1994; Breschi, Derosas and Manfredini, 2000; Corsini, 1991). This age range is supported by results found in statistical analysis of ecological factors and early life mortality in Northern Italy at this time (Breschi, Derosas, & Manfredini, 2004). While the study did not identify a link between living standards and infant mortality, there was a link demonstrated in mortality of children older than one year of age. This would indicate that the living conditions experienced by children within the first year of life were significantly less deleterious than those experienced by children after this age, which would align with the beginning of the weaning period.

The weaning process has several physiological consequences, including reduced nutritional intake and increased exposure to pathogens (Cummins and Thompson, 1997; Whitehead, 1985). The poor socioeconomic conditions of the Certosa children would have resulted in a particularly stressful weaning period. The preferred introductory food at this time was animal milk mixed with cornmeal or breadcrumbs (Hogan and Kertzer, 1987). In addition to being lower in nutritional value than breastmilk, this practice was regarded as unsafe by medical authorities due to the risk factors associated with the lack of bottle sanitation and issues of properly storing fresh milk (Hogan and Kertzer, 1987; Pozzi, 2002). This increased exposure to harmful bacteria or diseases is exacerbated by the loss of passive immunity which was provided through breast milk (McDade, 2003). A frequent result of this transition is gastroenteritis, often referred to as weaning diarrhoea in infants, which can lead to poor health, diminished growth, and increased risk of mortality (Pozzi, 2002; Temple et al., 2014). The effects of this can be seen in statistical data from the National Health Survey of 1885, which identified gastroenteritis as the leading cause of death for Italian children in the first years of life (Pozzi, 2002). For the children of the Certosa collection, a notable increase is seen in the number of deaths attributed to infectious or parasitic ailments, including gastroenteritis, after the age of one year. Approximately 18.5% of individuals died of these types of conditions prior to the age of one year, with that number increasing to 41.1% in the subsequent age category (Belcastro et al., 2017).

Further evidence for the high risk associated with the weaning process can be found in the mortality rate for Bologna's poorest children, as indicated by the age composition of the Certosa collection. Approximately one quarter of the 139 children included in the skeletal assemblage died between the ages of 1-2 years (Belcastro et al., 2017). In comparison, only 4% of the juvenile portion of the assemblage are individuals who died between the ages of 2-3 years. The relatively lower mortality during the second to third years of life indicate that the conditions experienced by children in the post-weaning period were more likely to result in their death than those experienced by children beyond this life stage. This disparity in mortality rates of infants in comparison to children is mirrored in regional data for Emilia-Romagna at the end of the 19th century, which show a large drop in mortality after the age

of 2 years (Del Panta and Pozzi, 2011). It should be noted that while the approximated age of weaning comes from both historical documents and statistical research on infant mortality in 19th century Northern Italy, there is a possibility that the actual age of weaning varies in the individuals of the Certosa collection. However, without the completion of isotopic analysis on the study sample, a more reliable age of weaning is not possible and interpretations should be considered in light of this.

As the TRD of the thoracic vertebrae complete development at approximately 1-2 years of age, it is likely that much of their growth would have occurred prior to the time of weaning, which may explain why this region was found to have the least amount of diminished growth. In comparison, the later developmental timelines of the cervical TRD and lumbar APD coincide with this stressful stage of life and extends past the infant buffering period, resulting in a greater amount of growth occurring in the weaning period. This would allow for the physiological effects of weaning and continued exposure to environmental risks to negatively impact canal growth in these dimensions. Although the conditions experienced by children after the age of 2 years seem to have improved, as evidenced by both the age demographic of the Certosa collection and historical records from this context, as this is the age at which the cervical TRD and lumbar APD begin completing fusion, it is unlikely that catch up growth would be possible in these dimensions, with too much of their growth having occurred and insufficient growth potential remaining. Therefore, the diminished growth resulting from the weaning period would then be locked in when fusion occurs. Conversely, the longer growth window of the lumbar TRD provides this diameter with a greater potential for catch-up growth in the years following the post-weaning period (Clark et al., 1988; Jeffrey et al., 2003).

The present interpretations regarding the impact of differing developmental timelines for the vertebral regions and accompanying exposure to biocultural factors would be strengthened by additional analysis of the thoracic vertebrae. As the thoracic region completes APD development later than both the cervical and lumbar vertebrae, with complete fusion not occurring until 5 years of age (Cunningham et al., 2017), the impact that

growth rate and potential for catch-up growth among the vertebral regions may be further understood through analyses of this dimension. It is apparent from the lack of available comparative literature that more research and data is needed to fully understand growth and stress response throughout the neural canal diameters of the spine. It is hoped that by beginning this line of inquiry into VNC development, other researchers will be inspired to do the same and that future studies will benefit from a more thorough approach to data collection of the vertebral column.

3.6. Conclusion

The aim of this study was to explore how the various regions of the developing spine record incidents of stress occurring during childhood. Through this research, it was intended to determine if the preferential focus on the lower vertebrae in studies of health in past populations was supported by the data. The results of this study demonstrate that diminished growth does not occur equally across the regions of the vertebral column, nor is it consistently found to be more severe in the lumbar vertebrae. Contrary to expectations, the cervical region was found to be significantly more reduced in growth in the transverse diameter than both the thoracic and lumbar regions. Comparison of the anterior-posterior diameter revealed that while the lumbar region was smaller for age relative to the cervical spine, these differences were not significant. These differences in growth reduction are thought to be associated with the varied developmental timelines of the spinal regions, where differing age of fusion and rate of growth alter the impact of growth stressors. These timing variations are accompanied by varying biocultural factors, which may impact growth in the vertebral elements, such as reduced nutrition intake and exposure to harmful bacteria following weaning. The findings of this study indicate that a focus on the lumbar region alone could overlook diminished growth and indications of early life stress recorded by those vertebrae developing on differing timelines. By including the cervical vertebrae in analyses of VNC, it is possible to identify growth insults which occurred before two years of age that otherwise might be missed or obscured due to catch-up growth if only the lumbar vertebrae are assessed.

4. Does age estimated from teeth forming in different early life periods show differential discrepancy with known age?

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4.1. Abstract

Objectives: The aim of this study is to explore growth discrepancies in the dentition of impoverished children and examine how dental development is impacted by environmental influences throughout childhood, thereby identifying which teeth are more sensitive to the effects of biocultural factors and are consequently less useful to predict age.

Methods: Length measurements of developing teeth (deciduous and permanent) were taken from individuals of known age and sex (n=61) from the Certosa collection, a 19th century skeletal assemblage representing Italian children of low socioeconomic status. Discrepancies between age estimates based on tooth length and chronological age were calculated, and the accuracy and precision of age prediction between earlier forming teeth and later forming teeth were compared.

Results: Deciduous teeth produced more precise dental age estimates (mean age discrepancy -0.092 years), while discrepancies between chronological age and age based on developing permanent dentition were larger (-0.628 years). The difference between these discrepancies in age estimates for deciduous and permanent teeth was significant ($p<0.001$), indicating that age prediction from deciduous tooth length is more accurate than age predicted using permanent tooth length.

Conclusion: An increasing variation and delay in tooth length for age reflects increasing susceptibility to biocultural factors, which impacts tooth growth during the course of childhood. Teeth whose development occurs earlier in life are less variable in their growth and provide more accurate estimations of age as a result.

4.2. Introduction

Although dental development has been found to be relatively stable and insensitive to biocultural factors, particularly in comparison to skeletal development, the sequence and timing of dental formation are not entirely immune to environmental influences (Cardoso 2007a; Conceição & Cardoso, 2011; Esan & Schepartz, 2019; May, Goodman, & Meindl, 1993). It is therefore possible that individuals who are exposed to adverse environmental and biocultural circumstances will experience a delay or stunting in dental growth that may be mediated by canalization. Although a well-acknowledged phenomenon, it is not known how the reduction of genetic control (Hall, 1999) and increasing variation in stress factors (Saunders, 2008) with age is manifested in the dentition, and therefore how the growth of teeth forming later in life differs from teeth which complete development earlier on. The aim of this study is to examine how growth in tooth height varies throughout dental development in both deciduous and permanent dentition. Although increasing variation in growth with increasing age has been exhibited in previous research on permanent teeth (Anderson, Thompson, & Popovich, 1976; Garn, Lewis, & Polacheck, 1959; Liversidge, 2009; Cardoso, Spake, & Liversidge, 2016; Šešelj, Sherwood, & Konigsberg, 2019), growth of the deciduous dentition has not been as thoroughly explored. This research aims to improve our understanding of how dental development is impacted by environmental influences throughout childhood. It will then be possible to determine which teeth are more sensitive to biocultural factors and are therefore less useful in estimating chronological age.

It is possible to identify deviations in dental growth by estimating age using tooth length and comparing this estimation to known chronological age. A deviation in growth is then identified as a discrepancy between estimated age and known chronological age. One of the simplest and most objective metric age assessment methods utilizing tooth length was developed by Liversidge and colleagues (1994; 1999). Using individuals from the known age Spitalfields collection, Liversidge and colleagues produced regression equations for age prediction from the length of an individual developing tooth. Recently, Cardoso and colleagues updated this method by doubling the sample size and altering the statistical method by employing an unbiased classical calibration approach (Cardoso, Spake, et al.,

2016; Cardoso, Meyers, et al., 2019). Many other methods of dental age assessment involve qualitative observations, utilizing comparative diagrams or atlases to assess the stage of crown or root formation, or the emergence of crowns in the oral environment (AlQahtani, Hector, & Liversidge, 2010; Demirjian, Goldstein, & Tanner, 1973; Moorrees, Fanning, & Hunt, 1963a, 1963b). Favoured for their simple and non-destructive applicability, these methods have the inherent drawback of user subjectivity. They are also limited in that continuous growth is confined to categorical stages. Quantitative age estimation techniques may therefore be more reliable due to increased objectivity and the ability to account for continuous tooth growth. Consequently, quantitative methods, such as tooth length, might produce more accurate and precise results than qualitative methods. The quantitative age estimation method using tooth length-for-age is also useful for identifying tooth growth deficits, as the increased precision makes it better able to identify smaller deviations from expected length than qualitative methods.

This study explores growth in developing dentition by examining a sample of documented child skeletal remains from the Certosa collection, a turn of the 20th century skeletal assemblage representing individuals of lower socioeconomic status from Bologna, Italy (Belcastro et al., 2017). Disruption in tooth growth was determined by assessing variation in age estimation from tooth length in the Certosa subadults using the equations from Cardoso and colleagues (Cardoso, Spake, et al., 2016; Cardoso, Meyers, et al., 2019) compared to known chronological age. Consequently, a difference in estimated age and known age would indicate a delay in tooth growth for age. It is predicted that diminishing genetic control over dental development, coupled with an exposure to accumulating and varying environmental stressors throughout childhood will be reflected as increasing variation in tooth length with age, along with an increasing delay in dental formation. Specifically, those teeth forming in utero and in the first postnatal years of life are expected to be less variable in length-for-age, whereas teeth forming later in childhood are anticipated to demonstrate increased variability, reflected as larger discrepancies between age estimated from tooth length versus chronological age. Therefore, those teeth that form during early life should predict age with greater accuracy than those teeth that form later in life, as the ability of teeth to predict age

accurately is a reflection of growth unimpeded by environmental effects. Furthermore, this research considers the biological mechanisms and pathways involved in dental development, in order to further our understanding of how biocultural influences can impact tooth formation.

4.3. Materials and Methods

The Certosa collection, currently housed at and curated by the Museum of Anthropology of Alma Mater Studiorum University of Bologna, Italy, comprises the skeletons of 425 impoverished individuals who died in Bologna between 1898 and 1944 (Belcastro et al., 2017). Both the juvenile and adult members of the collection are identified as being of low socioeconomic status (Belcastro et al., 2017), based primarily on their inhumation within the areas of the Certosa Cemetery traditionally reserved for Bologna's poorer classes (Vidor, 2012). For many of the adult members of the collection, as well as a handful of those in their late teens, this classification is supported by the occupation listed on their death certificates. Very few of the individuals had what would qualify as a skilled job, with most men showing employment as labourers, while the most commonly cited occupation for women was "housewife" (Belcastro et al., 2017). Additionally, previous research into the juvenile portion of the collection demonstrates stunted skeletal growth in comparison to both modern (Nelson, Harrington, Holland, & Cardoso, 2017) and contemporaneous historical samples (Cardoso et al., 2018). The study sample includes 61 individuals (37 females and 24 males) (Table 4.1), ranging in age from 40 days to 15 years, all but five of whom died in either 1900 or 1901. Associated birth and death records were obtained from civil archives and provide documented chronological age and sex for each individual. Age at death precision is to the day, with the exception of six individuals for whom records provided an age at death to the month. Mandibular deciduous and permanent teeth with a developing (incomplete) crown or root edge were selected for study, resulting in a sample of 279 teeth. A summary of teeth in the sample is included in Table 4.2, where the number of teeth for each tooth type is listed, as well as the mean number of deciduous and permanent teeth per individual. Maxillary teeth were excluded from this study as obtaining undistorted and accurate

radiographs of the maxillae can be problematic, and the age prediction equations are intended for mandibular teeth.

TABLE 4.1. Age and sex composition of the sample from the Certosa collection.

Age (years)	Total	Male	Female
< 1.0	16	5	11
1.0 – 1.9	21	8	13
2.0 – 2.9	5	2	3
3.0 – 3.9	2	1	1
4.0 – 4.9	2	1	1
5.0 – 5.9	5	1	4
6.0 – 6.9	1	-	1
7.0 – 7.9	4	2	2
8.0 – 8.9	1	-	1
9.0 – 9.9	1	1	-
10.0 – 10.9	-	-	-
11.0 – 11.9	2	2	-
12.0 – 12.9	-	-	-
13.0 – 13.9	-	-	-
14.0 – 14.9	1	1	-
Total	61	24	37

TABLE 4.2. Composition of the Certosa sample by tooth type, with mean number of deciduous and permanent teeth per individual.

	di1	di2	dc	dm1	dm2			Total Deciduous	Mean/Individual
n	16	25	31	33	33			138	3.15
	I1	I2	C	P3	P4	M1	M2	Total Permanent	Mean/Individual
n	30	27	14	16	14	35	5	141	2.49

Maximum tooth length was measured by taking the distance from the cusp or incisal edge to the developing edge of the crown or incomplete root, parallel to the long axis of the tooth (Figure 4.1) following Liversidge et al. (Liversidge, 1994; Liversidge & Molleson; 1999). Teeth with multiple cusps and roots (i.e., premolars and molars), were measured from the tallest

cusp to the longest developing root edge to capture maximum length. Only teeth with a well-preserved, developing crown or root (i.e., incomplete apex closure) were measured. Isolated teeth free from the jaws were measured using Mitutoyo callipers (Absolute Digimatic, Series 572) and recorded to the nearest hundredth of a millimetre. Measurements from developing mandibular teeth within their alveolar crypts were obtained from undistorted periapical radiographs using digital software. Radiographs were taken in the lingual-buccal plane with a 10mm bar providing scale. Radiographs were visually assessed to verify that the tooth had not rotated or tilted, and was in the correct plane. Any radiograph where teeth were clearly not in position was not used in this study. In order to assess whether radiographic measurements of tooth length differed from measurements of actual tooth length, a sample of 20 teeth was measured using both methods and analyzed for agreement using the Bland-Altman method (Bland & Altman, 1986). Additionally, measurement error in the isolated teeth was tested by comparing tooth lengths measured on two occasions, four weeks apart. A sample of 20 teeth was measured by one observer, and then re-measured by the first observer and a second observer. Following the method outlined by Ulijaszek and Kerr (1999), the relative technical error of measurement (%TEM) and coefficient of reliability (R) were calculated. Intra- and inter-observer error was assessed by comparing observations through a single calculation of %TEM and R.

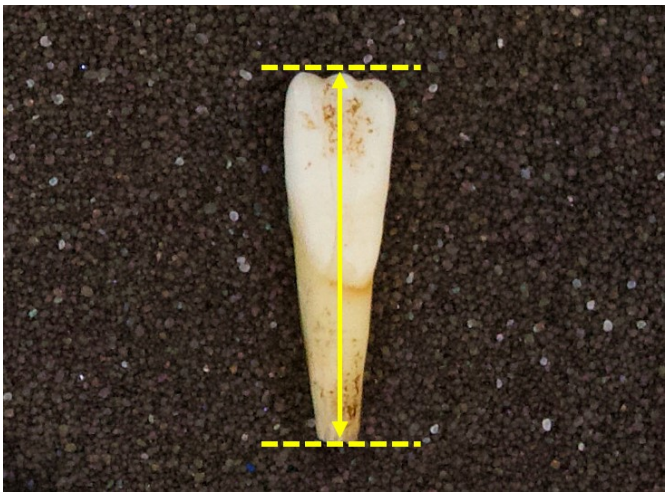

FIGURE 4.1. A visual of maximum tooth length; dashed yellow lines at upper (incisal edge) and lower (developing edge) limits; solid yellow line indicates parallel axis.

Growth patterns in tooth length-for-age were explored using two approaches. First, following Cardoso and colleagues (2016, 2019), ordinary least squares regression and classical calibration were used to generate age estimate formulae. Tooth length was regressed on the independent variable chronological age, for each tooth type. To evaluate the efficacy of age prediction in each tooth type, the regression coefficients from the Certosa sample were compared to those associated with the prediction formulae developed on the Lisbon and Spitalfields collections (Cardoso et al., 2016; 2019). The strength of the relationship in tooth-length-for-age across tooth types is determined by comparing the parameters for the classical calibration models, including sample size, min-max tooth length, and mean standard error (MSE) of ordinary least squares (OLS) regression of tooth length on chronological age for the Certosa sample, to the same parameters for the Lisbon and Spitalfields samples. MSE is the mean of standard errors calculated on each point as per Lucy (2005). Second, 'discrepancy' between the documented chronological ages of the Certosa children and the predicted age of each tooth, calculated using the sex-specific equations of Cardoso and colleagues (2016; 2019), was examined. These equations were considered appropriate as the tooth lengths from the Certosa collection fall within the required range for applicability. Age discrepancies were calculated by subtracting the documented age from the predicted age, such that a positive value represents an overestimation of chronological age, while a negative value represents an underestimation. The mean age discrepancy (MAD) and the mean absolute age discrepancy (MAAD) offer a measure of the accuracy and precision of age prediction for each tooth type, respectively. The MADs were compared to zero using one sample *t*-tests with an alpha-level of 0.05. To compare the accuracy of the deciduous and permanent dentitions, the MADs for all available deciduous and permanent teeth were compared using an independent samples *t*-test with an alpha-level of 0.05. In order to determine if discrepancies in age predictions between the samples are attributable to population differences in tooth size, tooth length for each tooth type were compared between Lisbon and Certosa for each developmental stage, assessed as per Moorrees, Fanning, & Hunt (1963a, 1963b). The Spitalfields collection was excluded from this analysis as tooth stage data were not available. Tooth lengths were compared using an independent

t-test for each tooth and stage with an alpha level of 0.05. The non-parametric Mann-Whitney U test was also used to assess differences in tooth length for stage and type between the two populations in order to compensate for issues related to small sample size.

4.4. Results

4.4.1. Tests of Measurement Error

The Bland-Altman test of agreement (1986) between measurements made on actual teeth versus radiographs shows that of the twenty pairs of measurements, nineteen fell within the limits of agreement (Figure 4.2) indicating there is no significant difference between the two methods. This is consistent with previous comparisons of radiographic and direct tooth measurements (Cardoso, 2007b; Liversidge & Molleson, 1999).

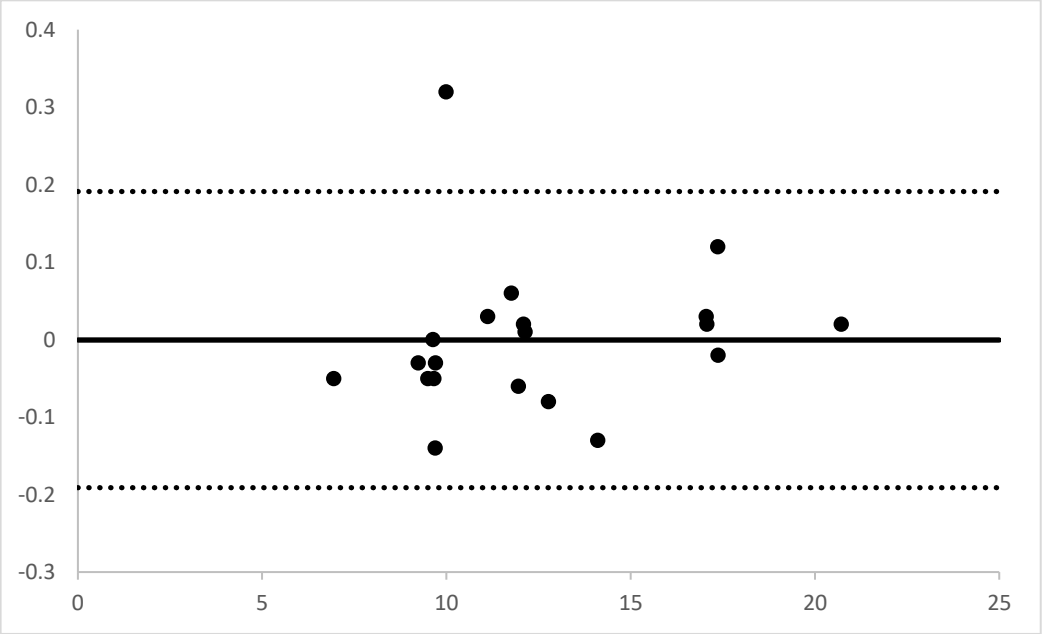

FIGURE 4.2. Bland–Altman plot of the difference between actual and radiographic length of 20 teeth. The dotted lines represent the upper and lower limits of agreement (1.96 standard deviations); the solid line represents the mean difference.

The results of the intra-observer and inter-observer error tests indicate that less than 1% of variation in the data is attributable to observer error (Table 4.3).

TABLE 4.3. Intra- and inter-observer error test results for tooth length measurements.

Intra-observer %TEM	R	Inter-observer %TEM	R
0.70	0.999	0.34	0.999

%TEM, relative technical error of measurement; R, coefficient of reliability.

4.4.2. Regression of Tooth Length on Documented Age

The Certosa coefficients of determination (R^2 values) are lower than the Lisbon and Spitalfields values, for all teeth except the permanent third premolar (P3) (Table 4.4). The MSEs are higher for the Certosa sample compared to those of Lisbon and Spitalfields, except for the permanent premolars (P3 and P4). In both samples, deciduous teeth have lower MSEs than permanent teeth; Certosa deciduous incisors and canines are similar in MSE to Lisbon & Spitalfields, but the MSE of deciduous molars and of the permanent teeth are higher in the Certosa sample. This is likely due to the high variance in Certosa residuals, evidenced by the high mean absolute residuals (MARs). In the Certosa sample, the actual age of the individual falls within the 95% prediction interval of the point estimate for all teeth except for the deciduous molars and first permanent incisor, where the percentage of individuals were between 93.94% for both deciduous molars and 93.33% for the first permanent incisor.

TABLE 4.4. Regression of tooth length on chronological age: Certosa in comparison with Lisbon & Spitalfields (2016; 2019).

	di1	di2	dc	dm1	dm2		
Lisbon & Spitalfields (Cardoso et al., 2019)							
n	40	56	69	65	85		
M	9.13	8.75	9.63	8.31	7.94		
SD	3.42	3.92	4.47	3.37	3.6		
Min–Max	3.30–15.04	2.87–15.43	1.80–18.63	3.23–13.90	2.00–14.55		
R^2	0.91	0.91	0.92	0.91	0.94		
MSE	0.19	0.21	0.29	0.25	0.27		
MR	0.00	0.00	0.00	0.00	0.00		
MAR	0.14	0.16	0.23	0.20	0.21		
% Range	97.5	94.64	95.65	96.92	95.29		
Certosa (this study)							
n	16	25	31	33	33		
M	10.60	10.10	8.43	8.27	8.13		
SD	1.90	2.56	2.78	2.59	2.46		
Min–Max	5.07–12.46	4.66–14.89	4.17–14.72	3.78–12.25	3.45–12.77		
R^2	0.80	0.83	0.87	0.78	0.75		
MSE	0.20	0.22	0.27	0.39	0.45		
MR	0.00	0.00	0.00	0.00	0.01		
MAR	0.76	0.89	0.63	1.01	1.01		
% Range	100.00	100.00	96.77	93.94	93.94		
	I1	I2	C	P3	P4	M1	M2
Lisbon & Spitalfields (Cardoso et al., 2016)							
n	77	79	92	67	51	100	53
M	9.44	9.35	9.50	9.06	11.00	8.04	12.06
SD	4.15	4.33	6.38	5.91	6.67	4.29	5.89
Min–Max	4.41–22.08	4.30–22.76	1.80–26.25	1.00–20.68	1.85–22.51	1.40–22.41	2.20–20.68
R^2	0.95	0.95	0.95	0.94	0.89	0.95	0.93
MSE	0.51	0.56	0.81	0.96	1.23	0.54	0.99

MR	0.00	0.00	0.03	0.00	0.02	-0.01	-0.01
MAR	0.38	0.42	0.57	0.75	0.86	0.42	0.72
% Range	94.06	93.68	93.48	94.03	94.12	96.00	96.23
Certosa (this study)							
n	30	27	14	16	14	35	5
M	9.28	10.2	10.2	8.16	9.77	8.24	7.96
SD	4.47	4.34	4.69	2.3	5.48	4.63	3.44
Min–Max	4.39–20.72	4.61–18.25	3.23–18.68	5.16–11.79	3.29–20.93	3.54–17.54	3.94–11.83
R^2	0.84	0.88	0.93	0.88	0.96	0.92	0.70
MSE	1.04	0.92	0.96	0.89	0.76	0.73	2.06
MR	0.00	0.00	0.01	0.00	-0.03	-0.01	0.03
MAR	1.20	1.10	0.92	0.63	0.91	1.01	1.30
% Range	93.33	100.00	100.00	100.00	100.00	97.14	100.00

M, mean tooth length (mm); SD, standard deviation for tooth length (mm); Min-Max, range of values for tooth length (mm); R^2, coefficient of determination from length regressed on age (length = a*age+b); MSE, mean standard error of point estimates; MR, mean residuals; MAR, mean absolute residuals; % Range, percentage of individuals who fall within 95% confidence interval.

4.4.3. Discrepancy Between Chronological and Dental Age

For the deciduous dentition, the overall mean age discrepancy (MAD) between estimated dental and known chronological age is -0.09 years, which ranged from an underestimation of 0.12 years for the second deciduous molar (dm2) to an overestimation of age by an average of 0.02 years for the central deciduous incisor (di1) (Table 4.5). The mean absolute age discrepancy (MAAD) is 0.18 years for the deciduous teeth, with a range of 0.13 to 0.25 years. One sample *t*-tests and Wilcoxon signed-rank tests with alpha-levels of 0.05 found that the age discrepancies do not differ from zero for the deciduous incisors and molars, meaning that the estimates are not statistically different from chronological age. Age estimations from the deciduous canines, however, tend to underestimate chronological age ($p = 0.039$).

TABLE 4.5. Summary statistics for discrepancy between estimated dental and chronological age and one sample *t*-test values for deciduous teeth.

Teeth	*n*	MAD	MAAD	SD	*t*	*P*
di1	16	0.02	0.13	0.165	-0.841	0.414
di2	25	-0.04	0.16	0.192	-0.936	0.359
dc	31	-0.07	0.19	0.271	131.50*	0.039*
dm1	33	-0.10	0.20	0.289	158.50*	0.050*
dm2	33	-0.12	0.25	0.334	176.50*	0.104*
All Deciduous	138	-0.092	0.18	0.289	−3.766	< 0.001

n, number of teeth; MAD, Mean Age Discrepancy; MAAD, Mean Absolute Age Discrepancy; SD, standard deviation of MAD; *t*, one sample *t* value for mean age discrepancies; *p*, p-value. Samples indicated with an asterisk (*) did not meet the assumptions of normality, therefore the non-parametric Wilcoxon signed-rank test was conducted instead.

For the permanent dentition, the MADs range from an average underestimation of 1.64 years for the permanent second molar (M2), to -0.04 years for the first molar (M1) (Table 4.6). The overall mean age discrepancy for the permanent teeth is -0.63 years. The MAADs for permanent teeth fall between 0.48 and 1.76 years, with the overall MAAD for permanent teeth being 0.55 years. One sample *t*-tests with an alpha-level of 0.05 found that age discrepancies differ significantly from zero in each permanent tooth type except for the first molar, suggesting statistically significant differences between chronological age and the estimated dental ages for all but one permanent tooth. Overall, discrepancies between estimated dental age and chronological age increase with teeth that form later in life (Figure 4.3).

TABLE 4.6. Summary statistics for discrepancy between estimated dental and chronological age and one sample *t*-test values for permanent teeth.

Teeth	*n*	MAD	MAAD	SD	*t*	*p*
I1	30	-0.46	0.69	0.165	-2.546	0.016
I2	27	-0.45	0.71	0.192	-3.199	0.004
C	14	-1.20	1.23	0.271	-4.742	< 0.001
P3	16	-1.10	1.13	0.289	-4.323	<0.001
P4	14	-1.07	1.20	0.334	-4.431	<0.001
M1	35	-0.04	0.48	0.975	-0.353	0.726
M2	5	-1.64	1.76	0.818	-3.081	0.037
All Permanent	141	-0.628	0.55	1.010	-7.320	< 0.001

n, number of teeth; MAD, mean age discrepancy; MAAD, mean absolute age discrepancy; SD, standard deviation of MAD; *t*, one sample *t* value for age discrepancies; *p*, p-value.

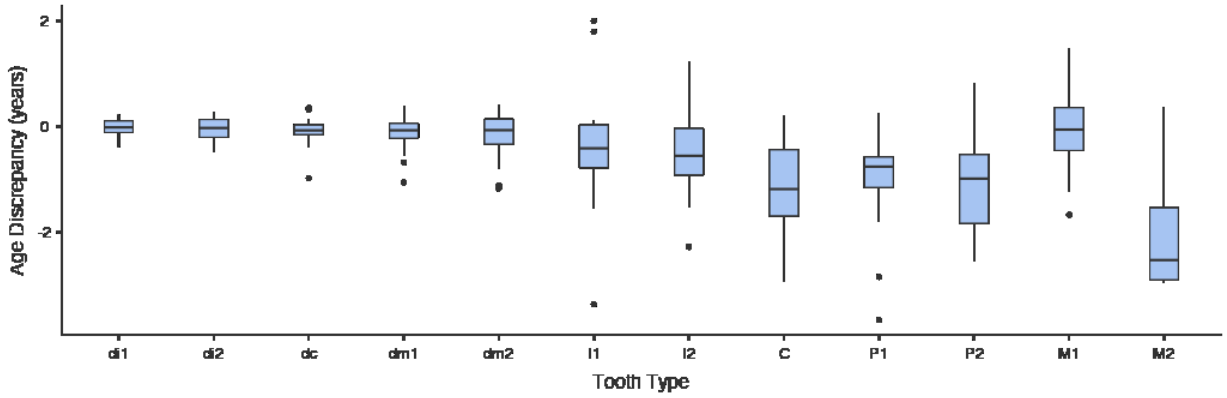

FIGURE 4.3. Boxplots showing the distribution of discrepancies between chronological and estimated dental age. The line in the box indicates the median, the box the inter-quartile range, and the whiskers the min-max.

An independent samples *t*-test shows the difference between the discrepancies in age estimations for the deciduous and permanent dentition is significant (Table 4.7), indicating that age predictions based on the length of deciduous teeth generally perform better than those from permanent teeth.

TABLE 4.7. Independent Samples *t*-test and Mann-Whitney U test for tooth length by tooth type and developmental stage for the Certosa and Lisbon collections.

		Certosa		Lisbon			M-W test	Ind. *t*-test
	Stage	n	Mean Length	n	Mean Length	Avg Diff	*p*	*p*
di1	10	6	11.45	2	11.62	-0.17	0.643	0.782
di2	6	2	5.38	2	5.51	-0.13	1.000	0.823
	9	5	11.89	2	9.68	2.21	0.095	0.057
dc	4	3	4.43	2	3.39	1.04	0.200	0.275*
	5	4	5.98	3	4.64	1.34	0.229	0.199
	6	8	7.06	2	7.39	-0.33	0.400	0.400*
	8	7	10.05	3	8.80	1.26	0.033	0.095
	9	3	13.15	2	12.42	0.73	0.400	0.339
dm1	5	3	5.37	2	4.27	1.11	0.200	0.058
	6	3	6.17	2	5.68	0.50	0.400	0.300
	9	5	10.71	2	8.03	2.69	0.095	0.002*
	10	3	11.80	4	11.00	0.81	0.629	0.366
dm2	4	3	4.90	3	4.41	0.49	1.000	0.576
	6	5	6.32	2	6.49	-0.17	1.000	0.715
	9	5	11.91	5	9.30	2.61	0.016	0.003

I1	5	3	6.79	4	6.63	0.16	0.629	0.874
	8	4	14.08	6	11.98	2.09	0.257	0.141
I2	5	5	7.17	3	6.99	0.18	0.786	0.810
	7	2	11.94	2	9.04	2.91	0.333	0.008
	8	3	13.35	5	12.51	0.84	0.393	0.626
	9	4	17.23	2	14.96	2.27	0.133	0.025
C	4	2	6.29	2	6.12	0.17	1.000	0.888
P3	6	4	8.42	2	7.74	0.68	0.800	0.673
P4	6	3	8.21	2	8.74	-0.53	1.000	0.704
M1	4	6	5.54	3	5.50	0.04	1.000	0.951
	5	3	7.66	2	5.61	2.05	0.200	0.141
	8	4	12.70	2	10.63	2.07	0.267	0.148
	9	4	14.29	2	11.44	2.85	0.133	0.023

Stage: developmental stage assessed as per Moorrees et al. (1963a, b); Mean Length: Mean Length in mm; Avg Diff: Average Difference in mm calculated by Certosa-Lisbon; *p*: p-value. Comparisons indicated with an asterisk (*) did not meet the assumptions of equal variances (Levene's test). Comparisons indicated in bold are statistically significant (p<0.005).

4.4.4. Lisbon vs. Certosa tooth length by developmental stage comparison

In total, 62 comparisons of mean tooth length for each tooth stage and type were completed. It was not possible to assess the stage of all teeth in the samples, and not every observation had a match in the other sample. The results of the independent samples *t*-tests for tooth length by tooth type and stage indicate that there are five situations where tooth

length is significantly different between the collections (Table 4.8). In all situations, Certosa has greater tooth length for stage than Lisbon. Cases where one of the samples had an *n* of one were removed, but have been retained in a supplementary table for clarity. There is one case where an *n* of one included a statistically significant difference. The supplementary table includes all *p*-values for the *t*-tests of all comparisons. Although the sample sizes for these comparisons are very small, the observed differences appear to be relatively consistent across all developmental stages and tooth types, suggesting the results are reliable and not due to inadequate sample size. A Mann-Whitney U test was conducted to determine if there were differences in tooth length between Certosa and Lisbon. In only one situation (dm2, stage 9) were there differences between the collections. Distributions of tooth length for the two collections were not similar as assessed by visual inspection; consequently, it is the rank that is compared and not the median. Tooth lengths for Certosa (mean rank = 7.8) were statistically significantly higher than for Lisbon (mean rank = 3.20, *U* =1.000, z = -2.402 p= 0.016), using an exact sample distribution for U, for dm2, stage 9. These results indicate that the Certosa collection has equal tooth length for dental stage in the majority of comparisons with Lisbon.

TABLE 4.8. Summary Statistics and Significance of Difference between Deciduous and Permanent Teeth

Deciduous Teeth			Permanent Teeth				
n	MAD	SD	*n*	MAD	SD	*t*	*p*
138	-0.092	0.289	141	-0.628	1.010	6.05	<0.001

Differential performance of deciduous vs. permanent teeth in age prediction in the Certosa sample. n, number of teeth; MAD, mean age discrepancy; SD, standard deviation of discrepancy; *t*, Welch's *t* value; *p*, p-value.

4.5. Discussion

In comparison to Lisbon and Spitalfields (Cardoso et al., 2016; 2019), the regressions in this study suggest higher variance in tooth length for the Certosa children. The greater variation may be due to smaller sample sizes per tooth in this study versus Lisbon & Spitalfields, as smaller samples suffer from the outsized effects of extreme values. Additionally, the large number of individuals below 2 years of age within our sample may impact the regressions as this age demographic is overrepresented, resulting in a poor fit with the predictive model.

Overall, greater variance is observed in later forming teeth in both the deciduous and permanent dentitions for Certosa, as evidenced by higher MSEs. The greater variance in later forming deciduous teeth could be a reflection of their differing developmental environments. The deciduous molars, and to some extent the deciduous canine, undergo less of their development in utero than the deciduous incisors, with the result being that they are exposed to different and greater sources of environmental stress, while the earlier forming teeth are buffered in comparison. This differs from what was found by Cardoso et al. (2016; 2019) who demonstrated fairly consistent MSEs across the deciduous dentition with only a slight increase in variation observed in the posterior teeth.

All of the permanent teeth, except the third premolar, show higher MSEs and lower R^2 values than those found by Cardoso and colleagues (2016). Although the results of that study did see higher levels of variance in the later forming permanent dentition, they observed much lower MSEs in the earlier forming permanent teeth (the incisors and first molar) in comparison to this study. Generally, the higher variation in later forming permanent teeth in Certosa compared to Lisbon and Spitalfields may suggest that the biocultural factors experienced in the months following birth were more deleterious to dental development in the Certosa sample context than in the Lisbon and Spitalfields sample contexts.

In addition to a greater variance in tooth length by age for later forming teeth, the results of this study indicate a delay in dental growth as evidenced by a consistent and increasing underestimation of chronological age. The tooth lengths from the Certosa sample fall within

the range of tooth lengths for the equations developed by Cardoso and colleagues (2016; 2019), therefore indicating that the equations should predict the chronological age of these individuals well, as such any difference between dental age and chronological age indicate a delay in tooth growth. In comparison to the permanent dentition, deciduous teeth more closely followed expected growth patterns, with MADs and MAADs far lower than those of the permanent teeth. These observations support the prediction that the effects of environmental influences on dental development with age results in an increase in variation in tooth length, which in turn causes less accurate and precise estimations of age. This larger variation is likely associated with an increased sensitivity of later forming teeth to environmental influences, such as those associated with the poor socioeconomic conditions experienced by these Italian children. These results are in line with previous findings (Cardoso et al., 2018) on the skeletal development for the Certosa sample, which was found to be more stunted in comparison to children from the Lisbon collection. From historical records, it is known that the children from the Certosa collection were born during a period of both political and social upheaval, following the unification of Italy just three decades prior in 1861 (Kertzer, Koball, & White, 1997). In addition, the rapid industrialization of Italy at this time led to an urban migration, resulting in a strain on Bologna's resources, leading to lower socioeconomic classes living in unhygienic and cramped housing (Hogan & Kertzer, 1987), while also surviving on poor diets and unsanitary well water (Drusiani et al., 2010; Faggioli, 2006; Tuttle, 2015). Furthermore, slightly different burial practices, which will be explained in more detail in subsequent paragraphs, may also have selected a poorer or more disadvantaged segment of the Bologna population in the Certosa sample (Belcastro et al., 2017) when compared to Lisbon (Cardoso et al., 2018).

The reduction in canalization with age (Hall, 1999) and the higher variability in growth associated with increasing age (Saunders 2008) support the observation that later forming teeth are potentially more susceptible to environmental stressors than those that form earlier, and which are under stronger genetic control. The reduction in genetic control on tooth development later in life therefore provides an opportunity for the effects of the environment to register. This pattern of increasing variation with increasing age is also

reflected in the earlier forming teeth of the permanent dentition. The first molar, along with the central and lateral incisors, produced the lowest MADs, as well as the smallest MAADs. As these are the first permanent teeth to undergo development (Smith, 1991), the greater precision and accuracy of age estimations based on their length is expected. In comparison to the later forming permanent teeth, such as the canine and premolars, the first molars and central and lateral incisors form earlier in childhood, with their development beginning approximately 6 months to 1 year post birth (Smith, 1991). The differential variation observed between the permanent first molar and incisors compared to the canine and premolars may be indicative of differences in the canalization of earlier and later forming teeth, as earlier and more rapid developmental processes are generally more highly canalized, and thus show greater stability in more adverse conditions (Hall, 1999). Earlier forming teeth may then be more buffered against environmental influences as a result, while the reduced canalization of the later forming teeth makes them more sensitive to these influences, with the potential for greater variation (Hall, 1999; Waddington, 1942). Additionally, it is possible that the circumstances experienced by children during this period, such as nursing and increased parental caregiving, sheltered them from environmental stressors, thereby protecting the development of teeth forming during this time. Conversely, the greater growth variations observed in the later developing dentition may be a result of the disadvantageous conditions experienced in later childhood or their accumulation over ontogeny, such as poor nutrition and increased exposure to adverse environmental conditions, resulting in the greater growth variations observed. These findings of variation in tooth length increasing with age are in line with previous research on tooth development (Anderson et al., 1976; Cardoso, 2007b, 2009; Cardoso et al., 2016; 2019; Garn et al., 1959; Liversidge, 2009; Šešelj et al., 2019).

Given that the Certosa tooth lengths fall within the required ranges, the age estimation equations from Cardoso and colleagues are expected to work well for Certosa sample. The subsequent underestimation of chronological age, which we ascribe to differences in environment, might on the other hand be suggested as being due to genetically based population differences in tooth size and development. While this is a possibility, existing

research indicates that observed differences between populations are primarily the result of environmental factors (Cardoso, 2009), with comparisons of samples from diverse geographic origins finding no significant differences in the timing of tooth formation (Elamin, Hector, & Liversidge, 2016; Liversidge, Speechly, and Hector, 1999; Maber, Liversidge, and Hector, 2006). Of the scarce studies that have examined population variation in tooth length specifically, one found only two teeth differed in size between geographically diverse samples (Smith, Wax, and Adler, 1989), while an analysis of chronologically diverse samples determined only the root length of one tooth differed (Smith et al., 1986). In addition, the results of the comparison of average tooth length for developmental stage indicate that the teeth in the Certosa sample do not differ significantly from the Lisbon sample in their length for stage in the majority of comparisons. If the greater variation observed in age predictions of the Certosa sample was the result of population differences in tooth size, it would be expected that the results of this analysis show that the populations differ in tooth length by developmental stage, this is not the case. Further to this point, if the observed differences in tooth development are due to population-specific genetic factors, then it would be logical to expect the delay to be present in all teeth. In this situation, each tooth would take the same time to develop, but would develop later in life when compared to available reference populations. However, in that case there would not be the pattern of increasing delay with age, as found in this study.

The higher variance and consistent underestimations of age produced by the permanent teeth of the Certosa sample suggest that dental development in the Italian children was more adversely affected by environmental factors present later in childhood, and that the sociocultural environment of Bologna at this time may have been poorer than that for the Lisbon and Spitalfields samples. In addition, the burial practices of the sample communities may also be a contributing factor. Although both the Certosa and Lisbon collections are composed of unclaimed cemetery remains from temporary graves, differences in their collection processes may have resulted in a greater representation of the most impoverished in the Certosa collection. Communal graves were commonly used in both the Bologna and Lisbon cemeteries in which the poorest segments of society were interred. While the use of

the communal grave was decommissioned in Bologna in 1850 (Vidor, 2012), this did not occur until 1962 (Samelo, 2015) for the cemeteries in Lisbon. As a result, the poorest individuals may have been removed from the pool of temporary graves that provided skeletal material to the Lisbon collection. The Certosa collection, on the other hand, includes the remains of society's poorest residents. Consequently, the children in the Certosa sample represent poorer children, whose mothers experienced higher stress and fewer resources, than those in the Lisbon Collection.

A common focus of previous anthropological research into the effects of environmental conditions on dental development has been evaluating it relative to skeletal growth and chronological age (Cardoso, 2007a; Conceição & Cardoso, 2011; May, Goodman, & Meindl, 1993; Šešelj, 2013). These studies established that while dental development is more resilient to the adverse effects of poor environmental conditions than skeletal development, there is still evidence it can be delayed or altered by related factors. Such research has laid the groundwork for a more in-depth examination of the biological mechanisms responsible for varied tooth formation and suggest that plasticity in dental development is greater than previously thought. Previous theories proposed in past research for these variations in dental development include genetics (Ubelaker, 1987; Lewis & Garn, 1960; May et al., 1993), hormones (Lewis & Garn, 1960; Edler, 1977; Garn, Lewis, & Blizzard, 1965), and age of formation (Šešelj, 2013). By drawing on clinical research, links can be made between biocultural influences and observed tooth length variations, such as those found in this study.

Over the past several decades, numerous gene proteins and signaling pathways responsible for controlling the process of tooth morphogenesis, have been identified (Chhabra et al., 2014; Jernvall & Thesleff, 2000). Wnt (Moriguchi, Yamada, Miake, & Nitta, 2011; Suomalainen & Thesleff, 2010; Yang et al., 2018), transforming growth factor (TGF) (Tummers & Thesleff, 2009; Yang et al., 2018), bone morphogenetic protein (Bmp) (O'Connell et al., 2012; Tummers & Thesleff, 2009), fibroblast growth factor (FGF) (Jarvinen et al., 2006; Tummers & Thesleff, 2009), and sonic hedgehog (Shh) (Jarvinen et al., 2006;

Tummers & Thesleff, 2009) are all signaling pathways that have been found to play regulatory roles during tooth formation. The abnormal functioning of any of these signalling pathways could result in developmental anomalies, in particular during the process of amelogenesis where their proper functioning is required for the secretion of enamel matrix by ameloblasts during the secretory stage of tooth development (Moriguchi et al., 2011; Yang et al., 2018). When the activation or inhibition of these pathways prevents normal ameloblast function during this stage, the extent of enamel formation is reduced or arrested (Tummers & Thesleff, 2009), the potential outcome of which could be delayed crown formation and reduced tooth length.

There have been many studies which explore possible biocultural factors involved in abnormal tooth development. Experimental work with animal and human models has examined the effects that nutrition, disease, and stress can have on ameloblast activity and the production of enamel. Research into calcium, boron, and vitamins C and D indicates each plays a role in the structure or production of enamel. For instance, animal and in vitro human studies have demonstrated the role that calcium has in ameloblast differentiation, organization, and activity, with enamel forming under hypocalcemic conditions being reduced and improperly mineralized (Bonucci, Lozupone, Silvestrini, Favia, & Mocetti, 1994; Chen, Zhang, Mendoza, & Den Besten, 2009; Nanci et al., 2000). Similar results were also found in animal studies on vitamin C (Shrestha, More, Keshwar, Shrestha, & Raut, 2019), boron (Haro Durand, Mesones, Nielsen, & Gorustovich, 2010), and vitamin D (Limeback et al., 1992), highlighting the various potential consequences a poor nutritional environment can have on tooth formation. Based on these findings, it is possible that the children within the Certosa sample experienced higher levels of malnourishment relative to those within the Lisbon and Spitalfields samples, as indicated by their delayed tooth growth. This is supported by historical accounts from the time, which described the nutritional level of the labour class living in Bologna as “terrible in every respect” (Hogan and Kertzer, 1987).

Experimental animal models have also been used to establish the effects of illnesses associated with fever on enamel formation. For example, an in vitro animal study by

Ryyanen et al. (2014) found that ameloblast activity was adversely affected during instances of high fever, as evidenced by defective or reduced enamel secretion. These findings are similar to those produced in research from Tung et al. (2006), who observed disturbances in the production of enamel in an experimental animal model involving fever induced rats. In addition, the effects of stress on tooth formation have been examined in studies discussing the impact of cortisol on the development of dental tissues. For instance, experimental work with rats has shown that cortisol hormones affect the activity of dental matrices and cells during the developmental process, potentially resulting in changes to the structural properties of enamel (Al-douri, Al-Salihi, & Mohammed, 2005). Furthermore, in their examination of social inequalities and oral health, Boyce et al. (2010) found that the higher levels of cortisol secretion in children from families of lower socioeconomic status may compromise the microanatomical structures of their teeth, linking cortisol reactivity with deleterious changes in enamel. The results of these studies suggest that not only could higher levels of illness and disease be present in the Certosa sample in comparison with the Lisbon and Spitalfields samples, but that higher levels of stress hormones may also be responsible for the observed differences in dental development. This is in keeping with the documented outbreaks of cholera and typhoid throughout this time period in Bologna (Drusiani et al., 2010; Faggioli, 2006), and is further supported by the cause of death for approximately 30% of individuals within the Certosa collection being attributed to infectious disease, such as gastroenteritis (Belcastro et al., 2017). Future research into the prevalence and distribution of enamel defects, specifically linear enamel hypoplasia, in the dentitions of the Certosa children will allow for a better understanding of the extent and ways in which their poor environment affected the development of their enamel.

Our understanding of how biocultural influences, such as the ones discussed here, are reflected in teeth will be increased by continuing to draw on existing research into the signaling pathways involved in amelogenesis. Furthermore, additional research into which physiological pathways are at work throughout the various stages and processes of tooth development will refine interpretations of biomarkers, allowing for improved knowledge of how environmental insults impact dental development throughout childhood. Specifically,

future studies into the signaling pathways and cellular activity involved in root formation are necessary, which while contributing to overall tooth length and therefore vital to a complete understanding of vertical tooth growth, are not yet fully understood (Li, Parada, & Chai, 2017). While it may not currently be possible to identify exactly which mechanisms are responsible for dental variations in archaeological contexts, interpretations of an individual's life history and environment based on their dentition will be improved through research of this nature.

4.6. Conclusion

Through the use of a documented skeletal collection, it was possible to assess variation in dental development and the subsequent effects of this on tooth-based age estimation methods. Those teeth forming earlier in life were found to be the least variable in their length, while increasing variation in tooth length was observed in the later forming teeth. By studying growth disruptions and variation in dental development, it is possible to gain information about early life experiences and growth environments beyond what is recorded in the more sensitive skeletal elements.

This research adds to existing methodological approaches by identifying those teeth which provide more precise and accurate estimations of age, and those which are more useful at indicating exposure to biocultural stressors during development. It can be expected that earlier forming teeth are the best indicators of age, however the same cannot be said for later forming teeth, unless the individual or individuals being assessed are not stressed. The findings of this work are not only applicable to the study of developmental environments of past populations, but also give insight into research on living children through the use of radiographs and provide evidence for age estimation methods relevant to forensic anthropology in which accurate age prediction is paramount.

By considering the biological mechanisms and pathways in dental development, there is the opportunity to increase our understanding of how tooth formation responds to the biocultural influences present in an individual's environment. Subsequently, this knowledge

has the potential to further our ability to interpret sociocultural circumstances through analysis of the dentition and make more appropriate methodological decisions about age estimation.

5. Discussion and Conclusion

5.1 Synopsis and Discussion of Research Findings

Over the past several decades, anthropologists have endeavoured to expand our knowledge of children and their experiences, both from a social and a physiological perspective. The resulting body of research has demonstrated the wealth of information that can be gained through the study of children, regarding not only their own experiences but also providing insight into the societal conditions which surround them (Halcrow and Tayles, 2008). Despite the advances that have been made in this field of study, its relatively recent origins have resulted in many areas that have yet to be fully explored (Mays, 2017). In particular, there is still much that is not understood regarding how an individual's biocultural environment impacts the development of their dentition and skeleton. As biological anthropologists rely on skeletal and dental indicators of early life stress in their study of past populations, adding to existing knowledge of how developing bones and teeth respond to various growth conditions will improve our ability to interpret these records of experiences in early life.

The intent of this research was to further current understanding of childhood development by examining ontogenetic systems through a biocultural lens. This was accomplished through two objectives:

1. To identify evidence of diminished growth across a range of developmental systems in the impoverished children of the Certosa Collection and interpret these findings within the biocultural context of 19th century Bologna, Italy.
2. To investigate whether observations of altered development in the study sample are in line with expectations based on several assumptions regarding patterns of diminished growth which are commonly seen in bioarchaeological research.

5.1.1 Experiences of the Certosa Children

To understand the developmental impacts of poverty and further our knowledge of what childhood was like for the poor of 19th century Italy, this dissertation analyzed the skeletal remains of children from the Certosa Collection. Born into impoverished families residing in Bologna, Italy in the late 1800s, these individuals ranged in age from birth to 18 years at the time of their deaths. This broad definition of the term child covers a lengthy developmental window, thereby allowing comparisons of physical growth and development across the different stages of early life, including infancy (0-3 years), childhood (3-7 years), juvenile (7-12 years), and adolescence (12-20) (Bogin, 2020). The various archival records associated with the collection give accurate chronological ages of these children, which in turn permit comparisons of dental and skeletal development with a level of precision that is not often possible in bioarchaeological research. This allows for accurate assessments of developmental disruptions through comparisons with age specific reference standards, thereby enabling consideration of age-related changes in the sociocultural realm. These archival records also provide the biological sex of each child. As there are currently no reliable methods of estimating the sex of pre-pubertal skeletal remains, known sex offers the unique opportunity to investigate the impact of gendered cultural practices on an individual's physical development.

The development of these children was assessed using three recognized biomarkers of an individual's growth status: long bone length, vertebral neural canal dimensions, and developing tooth length. Each of these anthropometric measurements represent a separate ontogenetic system including the appendicular skeleton, the axial skeleton, and the dentition. By incorporating several physical indicators, this research assesses varied evidence of an individual's lived experiences and their impact on developmental processes. This approach not only increases our understanding of variation present in the plasticity of these physiological systems, but also provides the opportunity to investigate how both biological and cultural factors change throughout early life history stages in this population.

Analyses of long bone length (Chapter 2) indicate that the linear skeletal growth of the Certosa children was negatively impacted by their biocultural conditions. When compared to updated

data from the Denver Growth Study (Maresh, 1943; 1955; Spake and Cardoso, 2021), the children in the study sample were significantly different in all diaphyseal lengths ($p < 0.01$) from the 'healthy' reference data. According to globally recognized parameters (WHO, 1995), the Certosa children are stunted in their growth, as demonstrated by a mean composite z-score (CZS) of ≤ -2 for the sample. Stunting is observed in the youngest age groups in the sample, however it is more prevalent in older individuals, with 75% of children over the age of 5 years having a mean CZS of ≤ -2, compared to 32% of individuals under the age of 2 years. When assessed individually, all diaphyseal lengths are stunted based on mean z-scores (≤ -2), except for the humerus (mean z-score = -1.47) and tibia (mean z-score = -1.81). Although mean z-scores for all bones are lower in males than in females, these differences are not significant ($p > 0.05$).

Similar findings were observed in analyses of the children's vertebral neural canal dimensions (Chapter 3). Using data taken from roentgenograms of living children (Hinck et al., 1962; Hinck et al., 1965; Hinck et al., 1966) as a reference standard, z-scores were calculated for both the anteroposterior and transverse diameters of the neural canal. Although z-scores were calculated using age and sex specific reference data, sexes were pooled in subsequent analyses to bolster against issues stemming from small sample sizes. As was found in the appendicular skeleton, these z-scores indicate that the axial development of the Certosa children was also adversely affected by factors related to their impoverished conditions. When assessed by vertebral region and diameter, 90% of individuals in the study sample produced a mean z-score of <-2 in a VNC diameter of at least one region. For the transverse diameter, mean z-scores were lowest in the cervical vertebrae (mean z-score = -2.19) and differed significantly from mean z-score values for the thoracic (mean z-score = -1.39) and lumbar (mean z-score = -1.54) vertebrae ($p \leq 0.001$). Of the two regions which could be evaluated for anteroposterior diameters, the lumbar vertebrae had the lowest mean z-scores (mean z-score = -2.46), however this did not differ significantly from the mean z-score for the cervical vertebrae in this diameter (mean z-score = -1.84, $p = 0.646$).

Examination of the children's dentitions provided further evidence of their poor living conditions (Chapter 4). Measurements of developing tooth length were taken from the Certosa individuals, from which estimations of biological age were calculated using equations created by Cardoso and colleagues (2016; 2019). The resulting age estimates were then compared with known chronological age at death. A negative discrepancy between documented chronological age and predicted age indicates disrupted or delayed tooth growth. Those teeth which undergo development earlier in life, including the deciduous dentition and first permanent molars, were found to be less variable in their growth than teeth forming later in life, producing age discrepancies which are not statistically different from chronological age ($p > 0.05$), except the deciduous canine ($p = 0.039$). The mean age discrepancy between estimated dental age and known chronological age for these teeth is -0.06 years. The remainder of the permanent dentition, all of which complete the majority of their growth following the weaning period, exhibited notable variation, with age estimates significantly different from chronological age ($p < 0.05$). The mean age discrepancy between estimated dental age and known chronological age for these teeth is -0.79 years.

When the results of these studies are considered collectively, interpretations can be made regarding varying biocultural conditions experienced at different points during early life. The findings of stunted diaphyseal lengths in individuals within the first year of life suggests that in addition to their own postnatal experiences of deleterious growth conditions, the skeletal development of the children may also have been impacted by poor maternal health and *in utero* stress. Given that the classification of the Certosa Collection as impoverished is partially based on parental socioeconomic status, findings which indicate the mothers of these children also suffered from poor biocultural circumstances is not unexpected. The possibility of poor maternal health is supported by the findings of consistently greater growth disruption in male children relative to female children. Although this observation is in line with existing research which has found males to be more susceptible to deleterious growth conditions than females (Stin, 1969; Stinson, 1985), it does not align with the gendered cultural practices of Northern Italy in the late 19th century. Census data from this time period cite lower rates of childhood disease and mortality in males, suggesting the existence of cultural practices which favoured

them over their female counterparts (Manfredini, Breschi, and Fornasin, 2017; Pinnelli and Mancini, 1999). Based on this information, it is likely that the variation in diaphyseal growth delays between the sexes began prior to birth, as the sex of the child would be unknown and gendered practices not possible. Due to their greater susceptibility to poor in utero conditions, the growth of male children would be more impacted by the time of their birth relative to female children. This would result in more severe growth delays in male children, which could not be corrected for, despite the preferential treatment they may have received postnatally.

Although there is evidence that long bone growth was delayed during *in utero* development, vertebral and dental elements developing during this period do not exhibit diminished growth. For instance, relative to the cervical and lumbar vertebrae, the transverse diameter of the thoracic vertebrae undergoes the majority of its development prior to birth (Cunningham et al., 2017). Unlike what was seen in the later forming vertebrae, the transverse diameters of the thoracic region were not found to be diminished in size, indicating growth conditions *in utero* did not result in developmental delays in vertebral canals. Similar findings were observed in the dentition, where those teeth which complete much of their development *in utero*, such as the deciduous dentition (Smith, 1991), were not found to have significant variation in length. These results may be indicative of varying levels of canalization across physiological systems (Waddington, 1942). Research into canalization indicates that those structures and systems essential to survival are under tighter developmental control and are therefore less sensitive to fluctuations in biocultural conditions (Gavin-Smyth and Ferguson, 2014; Hallgrimsson et al., 2012; Takahashi, 2018). This results in less variation being observed in these elements. As vertebrae and teeth are part of the vital neurological system, a high level of canalization is expected in their development (Zelditch et al., 2004). Malformation of neurological and dental tissues would severely threaten an individual's survival by impairing their cognitive development, locomotion, communication, breathing, and mastication, as is evident in the extremely high fetal mortality rates associated with neural tube defects (Copp & Greene, 2010). It is therefore understandable that development of vertebral and dental tissues would be buffered against stressors via the preferential allocation of resources over traits less crucial to survival, such as diaphyseal length (Boonekamp et al., 2017).

Further consideration of patterns in the severity and frequency of diminished growth indicate that the period of ontogeny associated with weaning was particularly stressful for the Certosa children. According to historical records from this time, women in Northern Italy typically weaned their children around 10-12 months after birth (Breschi and Livi Bacci, 1994; Breschi, Derosas and Manfredini, 2000; Corsini, 1991). The transition to weaning foods, such as cornmeal mixed with animal milk, would have limited the nutritional intake of the infant, while also exposing them to greater numbers of pathogens (Cummins and Thompson, 1997; Hogan and Kertzer, 1987; Pozzi, 2002; Whitehead, 1985), the potential outcomes of which include poor health, delayed growth, and increased risk of mortality (Pozzi, 2002; Temple et al., 2014). The evidence of this physiological stress is apparent in the varying levels of diminished growth observed in the vertebral neural canals. Although diameters which complete their growth prior to weaning were not found to be diminished in size, those diameters completing their development throughout the weaning period and undergoing fusion during the second and third years of life, specifically the transverse diameter of the cervical vertebrae and anteroposterior diameter of the lumbar vertebrae (Baker, 2005; Cunningham et al., 2017), were notably small relative to reference standards. The presence of weaning stress in this population is supported by an increase in the number of deaths attributed to infectious or parasitic ailments, such as gastroenteritis or weaning diarrhoea, in the Certosa Collection, where approximately 18.5% of infants under one year of age died due to these types of conditions, compared to over 41% of deaths in the subsequent age category of 1-6 years (Belcastro et al., 2017).

It is apparent from the findings of this research that the adverse circumstances experienced by these children did not cease following the weaning stage. Analyses of both appendicular and dental data suggest a worsening or accumulation of deleterious growth conditions in later childhood. This is demonstrated in the z-scores calculated from the diaphyseal lengths, where z-score values decrease with age, while the number of individuals qualifying as being stunted in growth based on their composite z-scores increases. Similar results were observed in the children's dental development, where variation was found to increase with age. This was evidenced by consistent and increasing underestimations of chronological age from teeth which

form later in life, including the permanent canines, premolars, and second molar. These data trends indicate that older children may have been exposed to more disadvantageous biocultural conditions than younger individuals or experienced the accumulating effects of a consistently poor growth environment, resulting in a greater amount of their developmental window being affected and severely impacting their potential for catch-up growth.

5.1.2 Patterns of Diminished Growth

This research endeavoured to refine current methodological approaches to the study of children and childhood development by evaluating several long-standing assumptions present in biological anthropology. The three scientific articles within this dissertation focus on different biomarkers commonly used in studies involving the archaeological remains of children, each of which has an associated assumption regarding expected patterns of diminished growth. The findings of these articles were comparatively evaluated against these expectations to assess if research results follow these assumptions, thereby testing their validity.

The first article examined the impact that developmental order and timing may have on the sensitivity of long bone development to perturbations in growth conditions. Development of the human body proceeds in cephalo-caudal and proximo-distal order, where elements and tissues located closer to the head and trunk develop earlier than those which are positioned farther away (Kingsbury, 1924; Scammon, 1923). This developmental pattern results in distal elements undergoing growth relatively later and at a more rapid rate than proximal elements, which has been linked with these elements having greater sensitivity to biocultural factors and consequently more likely to be stunted (Cardoso and Magalhaes, 2011; Bogin, 1999; Eveleth and Tanner, 1990; Smith and Buschang, 2004). Although this theoretical framework has led to preferential analysis of distal elements in bioanthropology research, namely the tibia (DeWitte, 2018; Lampl, Kuzawa, and Jeanty, 2003; Mensforth, 1985; Newman, Gowland, and Caffell, 2019), it has not been thoroughly explored. The intent of this article was to assess if growth stunting occurring in the study sample aligns with this pattern of differential variation in the sensitivity of growing long bone diaphyses. This study found that sensitivity was not consistent

with patterns expected based on developmental order. Although the distal segment of the upper limb was significantly more stunted in growth relative to the proximal segment, this was not the case in the lower limb, which was found to have significantly greater stunting in the proximal segment relative to its distal segment. These results are believed to be linked with varying developmental timelines of skeletal elements and the differing biocultural factors present throughout human life history stages, such as *in utero* development and infancy. Clinical research assessing the impact of maternal health status on fetal growth indicates that relative to both the humerus and the tibia, the femur undergoes more rapid rates of prenatal growth (Mehta and Singh, 1972; Neufeld et al., 2014), resulting in the femur having a relatively greater sensitivity to perturbations in the *in utero* growth environment (Bogin and Varela-Silva, 2010), aligning with research outcomes of the present study. The findings of this research article underscore the complexity of skeletal growth and plasticity, as well as demonstrating the information that can be gained by assessing growth of both proximal and distal skeletal elements.

The second article comparatively evaluated diminished growth in the dimensions of the vertebral neural canal in the three regions of the spine. The purpose of this study was to critically evaluate if current methodological practices which focus almost exclusively on lumbar vertebrae (Corron, Wolfe, and Stull, 2021, 2023; Papp, Porter, and Aspden, 1994; Porter and Pavitt, 1987b; Rewekant, 2001; Watts, 2011, 2013a, 2013b, 2015) are correct in their assumptions that this region is the most likely to record evidence of growth disruption. The reasoning behind this assertion has been cited as the lengthier developmental window of the lumbar vertebrae, which in turn is considered to provide more opportunity for exposure to biocultural stressors (Clark et al., 1985; Watts, 2011). While the majority of bioanthropological research involving vertebral neural canal measurements include only lumbar vertebrae, the validity of this methodological approach has yet to be demonstrated. This study found that while the lumbar vertebrae were smaller than the cervical vertebrae in the anterior-posterior diameter, this difference was not significant (p=0.148). Results for the transverse diameter analysis found that the cervical vertebrae were significantly more diminished in the growth of this dimension than both the thoracic ($p \leq 0.001$) and lumbar vertebrae ($p \leq 0.001$), which did

not differ significantly from each other (p = 0.524). Consequently, the prevailing belief that lumbar vertebrae are the most useful for detecting episodes of stress and growth disruption is not supported by these study findings. These results reveal that existing research approaches which exclude the earlier developing cervical and thoracic vertebrae may be overlooking evidence of early life stress. To the best of the author's knowledge, this represents the first study which comparatively assesses vertebral neural canal growth between the spinal regions.

The third article explored variation in dental development to assess how tooth formation responds to biocultural conditions throughout childhood. Although research has established that the development of the dentition is relatively more resilient to environmental perturbations than skeletal development (Cardoso, 2007a; Conceição and Cardoso, 2011; Garn, Lewis, and Blizzard, 1965; Lewis and Garn, 1960; Šešelj, 2013; Ubelaker, 1987), evidence indicates that the sequence and timing of dental formation are not wholly impervious to adverse growth conditions (Cardoso, 2007a; Conceição and Cardoso, 2011; Esan and Schepartz, 2019; May, Goodman, and Meindl, 1993; Šešelj, 2013). In spite of this knowledge, bioanthropological research largely assumes that age estimations based on an individual's dental development provide an accurate indicator of their chronological age, with no recognition of potential errors or biases found in recent research (Blom et al., 2021; Hodson and Gowland, 2020; Newman, Gowland, and Caffey, 2019). This study sought to determine the extent to which tooth development and age estimations based on the dentition are impacted by biocultural stressors. Study results reveal variance in tooth length for age in both the deciduous and permanent dentition of the Certosa children. These findings add to the body of research demonstrating that tooth formation is altered or delayed by poor environmental circumstances. Teeth which form earlier in life were found to be less variable in their growth than teeth undergoing formation later in childhood. This was considered to be reflective of varying genetic control and exposure to stress factors. These results indicate that age estimations are more accurate when calculated using earlier forming teeth, while later forming teeth may be more useful for detecting experiences of stress exposure. Studies which rely on estimations of age based on dental development should be cognizant of these patterns when considering research design and data interpretation.

The findings of these articles demonstrate the need to continue testing and questioning our knowledge, particularly when it comes to the study of children. These observations reveal that there is still much that remains unknown regarding developmental plasticity and stress responses during early life. The results of this dissertation highlight the complexity of growth processes and biocultural variation in development.

5.2 Future Research Directions

This research focused on the experiences of children from Bologna's impoverished families at the end of the 19th century. To gain a more complete understanding of the lives of these individuals, future studies which centre around the adult members of the Certosa Collection may add to the knowledge obtained through this doctoral project. Like the children within the collection, the Certosa adults (defined as 18 years in age or older) represent a relatively narrow temporal context. Approximately 80% (n = 203) of the adult segment was born between 1850 and 1909, with one third of these individuals sharing the same birth window as the Certosa children (Belcastro et al., 2017). Although few direct familial relationships exist in the collection, many of the adult individuals represent a comparable growth cohort to parents of the Certosa children. Therefore, the study of their physical remains has the potential to add further contextual data regarding the home life of the Certosa children by providing information related to the nutrition and illness of the adult members of Bologna's lower socioeconomic class. These individuals would have been exposed to differing and perhaps greater biocultural stressors than the younger members of society, which then may have impacted their children via contact within their homes or during gestation. This examination of adult experiences would permit a more holistic understanding of what life was like for the Certosa children and offer greater insight into the biocultural factors responsible for their developmental deviations. For instance, evaluating the nutritional status of the female adults would provide further evidence pertaining to maternal health, as would exploring their ages and cause of death. This information would strengthen interpretations of *in utero* conditions and physiological development for the study sample.

By studying those adults born between 1890 and 1909 (n = 70), comparative insight can be gained into the developmental outcomes of individuals who survived childhood relative to those who died before reaching adulthood (Belcastro et al., 2017). These comparisons would further our understanding of living conditions for Bolognese children at this time through the analysis of ontogenetic evidence of childhood experiences observable in adult remains, such as dental and vertebral biomarkers. This type of research offers the opportunity to gain information of early life history stages from individuals who may have had differing experiences of childhood than those who died prematurely. As individuals within the Certosa Collection lived and died during a relatively narrow window of time, it is possible to evaluate how biocultural conditions from this context may have differentially affected those who succumbed to their impoverished circumstances with those who survived. Research of this nature would provide additional information regarding the lives and experiences of impoverished children in Bologna at the end of the 19th century.

To date, there have been no isotopic analyses performed on the Certosa Collection. This avenue of research would add valuable contextual data to interpretations which focus on the biocultural environment experienced by these individuals. In particular, analysis of stable isotopes, such as nitrogen, carbon, and oxygen, in the hard tissues of bones and teeth would shed light on the accuracy of historical accounts of weaning practices from this time period (Jay, 2009). This information would strengthen interpretations of dental and skeletal indicators of stress which are believed to be the result of the weaning transition, as well as provide information regarding the experiences of impoverished mothers in urban centres at the turn of the 19th century in Italy. Analysis of these stable isotopes in male and female children would also provide additional data regarding gendered cultural practices in Northern Italy at this time. By comparatively studying evidence of the diets of male and female children, stronger interpretations can be made pertaining to preferential treatment of boys at this time. In turn, this may provide supporting data for maternal health and impacted *in utero* development, as evidence which contradicts the favouring of male children would suggest that growth deviations occurred prior to birth.

5.3 Conclusions

Despite making up a large proportion of society, the lives and experiences of children have often been overlooked in research of past populations. As a result, our knowledge of what childhood was like in the past is quite limited, as is our understanding of how ontogeny is impacted by varying biocultural conditions throughout early life history stages. This thesis sought to fill these gaps in the literature through analysis of the skeletal remains of children from impoverished families in Bologna, Italy at the end of the 19th century. By investigating physical evidence of these children's lives along with historical records of their sociocultural environment, this research was able to evaluate biological evidence within the appropriate cultural context, recognizing the biocultural nature of human development.

The results of this research demonstrate that the adverse circumstances experienced by the children of the Certosa Collection negatively impacted the development of their appendicular, axial, and dental systems. Analysis of varied biomarkers indicate that the effects of their poor socioeconomic status began prior to their birth, with evidence of poor maternal health and diminished or delayed *in utero* growth present in the youngest individuals in the study sample. The differential patterns of affected growth observed in the dental and skeletal indicators suggest that following a relatively sheltered period of parental care during infancy, these children suffered from substantial physiological stress once weaned. It is apparent from the increasing frequency and severity of altered growth found in these studies that intensifying or accumulating deleterious biocultural factors were experienced throughout childhood.

Consideration of existing bioanthropological research involving the remains of children reveals several long-standing assumptions pertaining to developmental processes, which have influenced the methodological approaches employed in many publications, as well as their subsequent interpretations of childhood experiences. This thesis comparatively evaluated the indicators of diminished growth observed in the Certosa children against expected patterns based on these assumptions to assess their validity. Findings from all three studies deviated from these expectations, indicating there is still a need for ongoing research to understand the

levels of complexity present in early human development. These results demonstrate the need to remain critical and curious in our research.

The three studies included in this dissertation reveal what life was like for impoverished Bolognese children at the turn of the 20th century. These findings further our knowledge beyond this specific sociocultural context by adding to the overall body of literature on the experiences of childhood. Through this research, a better understanding of how various ontogenetic systems respond to biocultural conditions in early life was attained. These advancements will allow for more informed methodological decisions in future bioanthropological studies of children, which in turn will improve our interpretations of their lives and the societies in which they lived.

6. References

Abe T, Sato T, Yoda T, et al. (2019). The period circadian clock 2 gene responds to glucocorticoids and regulates osteogenic capacity. *Regen Ther*, 11, 199–206.

Adriani, M., Wirjatmadi, B. (2014). The effect of adding zinc to vitamin A on IGF-1, bone age and linear growth in stunted children. *J. Trace Elem. Med Biol*. 28 (4), 431–435. doi: 10.1016/j.jtemb.2014.08.007.

Al-douri, A., Al-Salihi, A., & Mohammed, S. (2005). Glycoconjugates cytochemistry in teeth development in normal and hydrocortisone treated rats (experimental study). *Journal of Baghdad College of Dentistry*, 17(3), 70–75.

Albertsson-Wikland K, Karlberg J. (1997). Postnatal growth of children born small for gestational age. *Acta Paediatr*; Suppl 423:193-5. Stockholm. ISSN 0803-5326

Allen, M. R., & Burr, D. B. (2019). Chapter 5 - Bone Growth, Modeling, and Remodeling. In M. R. Allen & D. B. Burr (Eds.), *Basic and Applied Bone Biology, Second Edition* (pp. 85-100). Academic Press. doi: 10.1016/B978-0-12-813259-3.00005-1

AlQahtani, S., Hector, M., & Liversidge, H. (2010). Brief communication: The London atlas of human tooth development and eruption. *American Journal of Physical Anthropology*, 142(3), 481–490. doi: 10.1002/ajpa.2125

Aly, T. & Amin, O. (2013). Geometrical Dimensions and Morphological Study of the Lumbar Spinal Canal in the Normal Egyptian Population. *Orthopedics*, 36(2), 229–234. doi: 10.3928/01477447-20130122-27

Amonoo-Kuofi, H. S. (1982). Maximum and minimum lumbar interpedicular distances in normal adult Nigerians. *Journal of Anatomy*, 135(Pt 2), 225–233.

Amonoo-Kuofi, H. S. (1985). The sagittal diameter of the lumbar vertebral canal in normal adult Nigerians. *Journal of Anatomy*, 140(Pt 1), 69–78.

Amoroso, A., & Garcia, S. J. (2018). Can early-life growth disruptions predict longevity? Testing the association between vertebral neural canal (VNC) size and age-at- death. *International Journal of Paleopathology*, 22, 8–17.

Anderson, D. L., Thompson, G. W., & Popovich, F. (1976). Age of attainment of mineralization stages of the permanent dentition. *Journal of Forensic Sciences*, 21, 191–200. doi: 10. 1520/JFS10353J

Auerbach BM, Sylvester AD. (2011). Allometry and apparent paradoxes in human limb proportions: implications for scaling factors. *Am J Phys Anthropol* 144:382-391.

Bagnall, K.M., Harris, P.F. and Jones, P.R.M. (1977). A radiographic study of the human fetal spine. 2. The sequence of development of ossification centres in the vertebral column. *Journal of Anatomy* 124: 791–802.

Baker, B., Dupras, T., & Tocheri, M. (2005). The Osteology of Infants and Children. Texas A&M University Press

Balic, A. (2019). Concise Review: Cellular and Molecular Mechanisms Regulation of Tooth Initiation, *Stem Cells*, 37(1), 26–32, doi: 10.1002/stem.2917

Baranova, J., Büchner, D., Götz, W., Schulze, M., & Tobiasch, E. (2020). Tooth Formation: Are the Hardest Tissues of Human Body Hard to Regenerate? *International Journal of Molecular Sciences*, *21*(11), 4031. doi: 10.3390/ijms21114031

Barker, D. J. P. (2012). Developmental origins of chronic disease. *Public Health, 126*(3), 185–189. doi:10.1016/j.puhe.2011.11.014.

Bartlett, J. D. (2013). Review Article: Dental Enamel Development: Proteinases and Their Enamel Matrix Substrates, *ISRN Dentistry*, 1, 1-24, doi: 10.1155/2013/684607

Bass, S., Delmas, P., Pearce, G., Hendrich, E., Tabensky, A., & Seeman, E. (1999). The differing tempo of growth in bone size, mass, and density in girls is region specific. *Journal of Clinical Investigation*, 104, 795– 804.

Baxter, J. E. (2008). The Archaeology of Childhood. *Annual Review of Anthropology*, *37*(1), 159–175. doi: 10.1146/annurev.anthro.37.081407.085129

Belcastro, M., Bonfiglioli, B., Pedrosi, M., Zuppello, M., Tanganelli, V., & Mariotti, V. (2017). The History and Composition of the Identified Human Skeletal Collection of the Certosa Cemetery (Bologna, Italy, 19th-20th Century). *International Journal Of Osteoarchaeology*, *27*(5), 912-925. doi: 10.1002/oa.2605

Betts, J. G., K. A. Young, J. A. Wise, E. Johnson, B. Poe, D. H. Kruse, O. Korol, J. E. Johnson, M. Womble and P. DeSaix (2013). Bone tissue and the skeletal system. In. *Anatomy and Physiology*. OpenStax Copyright 2020 by the Creative Commons Attribution License v4.0.

Betts, P.R., Magrath, G. (1974) Growth pattern and dietary intake of children with chronic renal insufficiency. *BMJ* 2:189–193

Bhattacharya, N. and Stubblefield, P.G. (2016). *Human Fetal Growth and Development: First and Second Trimesters* (N. Bhattacharya and P.G. Stubblefield, Eds.). Springer. ISBN : 978-3-319-14873-1

Bick, E.M., Copel, JW. (1950). Longitudinal growth of the human vertebra: a contribution to human osteology. *J Bone Joint Surg* 32:804-814.

Black, R.E., Allen, L.H., Zulfiqar, A.B., Caulfield, L.E., de Onis, M., Ezzati, M., Mathers, C., and Rivera, J. (2008). Maternal and child undernutrition: global and regional exposures and health consequences. *Lancet*, 371, 243-260. doi:10.1016/S0140- 6736(07)61690-0

Bland, J. M., & Altman, D. G. (1986). Statistical methods for assessing agreement between two methods of clinical measurement. Lancet, 327(8476), 307–310. doi:10.1016/ S0140-6736(86)90837-8

Bleuze, M.M., Wheeler, S.M., & Williams, L.J. (2023). Skeletal Growth Status in a Physically Abused Child from the Kellis 2 Cemetery, Egypt, *Childhood in the Past*, 16:1, 13-35, doi: 10.1080/17585716.2022.2036297

Blom, AA, Schats, R, Hoogland, MLP, Waters-Rist, A. (2021). Coming of age in the Netherlands: An osteological assessment of puberty in a rural Dutch post-medieval community. *Am J Phys Anthropol*, 174: 463–478. doi: 10.1002/ajpa.24161

Bogin, B. (1999). *Patterns of Human Growth*. Cambridge University Press.

Bogin, B., & Varela-Silva, M. I. (2003). Anthropometric Variation and Health: A Biocultural Model of Human Growth. *Journal of Children's Health*, 1(2), 1-24.

Bogin, B., & Varela-Silva, M. I. (2010). Leg length, body proportion, and health: A review with a note on beauty. *International Journal of Environmental Research and Public Health*, 7, 1047–1075.

Bogin, B., Smith, P., Orden, A. B., Varela Silva, M. I., and Loucky, J. (2002). Rapid change in height and body proportions of Maya American children. *American Journal of Human Biology*, 14, 753–761.

Bonucci, E., Lozupone, E., Silvestrini, G., Favia, A., & Mocetti, P. (1994). Morphological studies of hypomineralized enamel of rat pups on calcium-deficient diet, and of its changes after return to normal diet. *The Anatomical Record*, 239(4), 379–395. doi:10.1002/ar.1092390405

Boonekamp, J.J., Dijkstra, R., Dijkstra, C. and Verhulst, S. (2017), Canalization of development reduces the utility of traits as fitness biomarkers: feather fault bars in nestling birds. *Funct Ecol*, 31: 719-727. doi:10.1111/1365-2435.12765

Boyce, W., Den Besten, P., Stamperdahl, J., Zhan, L., Jiang, Y., Adler, N., & Featherstone, J. (2010). Social inequalities in child- hood dental caries: The convergent roles of stress, bacteria and disadvantage. *Social Science & Medicine*, 71(9), 1644–1652. doi:10.1016/j.socscimed.2010.07.045

Brandt, I. (1978) Growth dynamics of low-birth weight infants with emphasis on the perinatal period. In: *Human Growth and Postnatal Growth*/ F. Falkner and J.M. Tanner (eds.)/ Plenum Press, New York pp. 557-617.

Brandt, I. (1982) Human Growth up to Six Year – Distance and Velocity. In: *Genetic and Environmental Factors During the Growth Period*/ C. Susanne (ed.)/ Plenum Press, New York pp. 101-122.

Breschi M., and Livi Bacci, M., (1994). Le mois de naissance comme facteur de survie des enfants. *Annales de démographie historique*, pp. 169-185. doi: 10.3406/adh.1994.1865

Breschi, M., Derosas, R., & Manfredini, M. (2004). Mortality and environment in three Emilian, Tuscan, and Venetian communities, 1800–1883. In T. Bengtsson, C. Campbell, & J. Z. Lee (Eds.), *Life under pressure: Mortality and living standards in Europe and Asia, 1700–1900* (pp. 209–251). Cambridge: MIT Press.

Breschi, M., Fornasin, A., Manfredini, M., Mazzoni, S., Pozzi, L. (2011). Socioeconomic conditions, health and mortality from birth to adulthood, Alghero 1866-1925. *Explor. Econ. Hist*. 48(3), 366–375. doi:10.1016/j. eeh.2011.05.006.

Bromley B, Brown DL, Benacerraf BR. (1993). Short femur length associated with severe intrauterine growth retardation. *Prenat Diagn* 13: 449–452.

Brooke, O., Wood, C., & Butters, F. (1984). The body proportions for small-for-dates infants. Early Human Development, 10(1-2), 85-94. doi: 10.1016/0378-3782(84)90114-2

Buikstra JE, Ubelaker DH. (1994). Standards for data collection from human skeletal remains. *Fayetteville: Arkansas Archeological Survey Research Series* No. 44.

Buschang PH. (1982). Differential long bone growth of children between two months and eleven years of age. *Am J Phys Anthropol* 58:291–295.

Cameron, N. (2012). *Human Growth and Development*. Academic Press.

Cameron, N., Tanner, J.M. & Whitehouse, R.H. (1982) A longitudinal analysis of the growth of limb segments in adolescence, *Annals of Human Biology*, 9:3, 211-220, doi: 10.1080/03014468200005701

Cardoso HFV. (2005). Patterns of growth and development in relation to environmental quality. A biocultural analysis of a sample of 20th century Portuguese subadult documented skeletons. PhD Thesis. McMaster University, Hamilton.

Cardoso, H. F. V. (2007a). Environmental effects on skeletal versus dental development: Using a documented subadult skeletal sample to test a basic assumption in human osteological research. *American Journal of Physical Anthropology*, 132(2), 223–233. doi:10.1002/ajpa.20482

Cardoso, H. F. V. (2007b). Accuracy of developing tooth length as an estimate of age in human skeletal remains: The deciduous dentition. *Forensic Science International*, 172(1), 17–22. doi:10.1016/j.forsciint.2006.11.006

Cardoso, H.F.V. (2008a). Epiphyseal fusion at the innominate and lower limb in a modern Portuguese skeletal sample, and age estimation in adolescent and young adult male and female skeletons. *Am. J. Phys. Anthropol*. 135, 161–170.

Cardoso, H.F.V. (2008b). Age estimation of adolescent and young adult male and female skeletons. II. Epiphyseal union at the upper limb and scapular girdle in a modern Portuguese skeletal sample. *Am. J. Phys. Anthropol*. 137, 97–105.

Cardoso, H. F. V. (2009). Accuracy of developing tooth length as an estimate of age in human skeletal remains: The permanent dentition. *The American Journal of Forensic Medicine and Pathology*, 30(2), 127–133. doi:10.1097/paf. 0b013e3181879d07

Cardoso, Hugo F. V., and Teresa Magalhães. (2011). “Evidence of Neglect from Immature Human Skeletal Remains: An Auxological Approach from Bones and Teeth.” In The Juvenile Skeleton in Forensic Abuse Investigations, edited by Ann H. Ross, and Suzanne Marie Abel, 125–150. New York: Humana Press.

Cardoso, H.F.V., Abrantes, J. & Humphrey, L.T. (2014). Age estimation of immature human skeletal remains from the diaphyseal length of the long bones in the postnatal period. *Int J Legal Med* 128, 809–824 (2014). doi:10.1007/s00414-013-0925-5

Cardoso, H. F. V., Spake, L., & Liversidge, H. (2016). A reappraisal of developing permanent tooth length as an estimate of age in human immature skeletal remains. *Journal of Forensic Sciences*, 61(5), 1180–1189. doi:10.1111/1556-4029.13120

Cardoso, H.F.V., Harrington, L., Nelson, J., Holland, E., Tanganelli, V., & Belcastro, M.G. (2018). Are Social Inequalities Embodied as Growth Disparities in Children? Poster session presented at the 87th Annual Conference of the American Association of Physical Anthropologists. Austin, TX. doi: 10.1002/ajpa.23489, 165, 1, 312

Cardoso, H. F. V., Meyers, J., & Liversidge, H. (2019). A reappraisal of developing deciduous tooth length as an estimate of age in human immature skeletal remains. *Journal of Forensic Sciences*, 64(2), 385–392. doi:10.1111/1556-4029.13892

Cesar, A., Grkman, M., & Medic, M. (2020). Magnification Error in Radiographs of Cervical Spine in Lateral Projection. *Medical Imaging and Radiotherapy Journal*, 37, 15-19.

Chen, J., Zhang, Y., Mendoza, J., & Den Besten, P. (2009). Calcium-mediated differentiation of ameloblast lineage cells in vitro. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution*, 312B(5), 458–464. doi:10.1002/jez. b.21279

Chhabra, N., Goswami, M., & Chhabra, A. (2014). Genetic basis of dental agenesis - molecular genetics patterning clinical dentistry. *Medicina Oral Patología Oral y Cirugia Bucal*, 19(2), e112–e119. doi:10.4317/medoral.19158

Cianfarani, C. Ladaki, & C. Geremia (2006). Hormonal Regulation of Postnatal Growth in Children Born Small for Gestational Age. *Hormone Research* 65 (Suppl. 3): 70–74.

Cirillo, F., Lazzeroni, P., Sartori, C., & Street, M.E. (2017). Inflammatory Diseases and Growth: Effects on the GH-IGF Axis and on Growth Plate. *International Journal of Molecular Sciences* 18(9): 1-19. doi:10.3390/ijms18091878

Clark, G. (1985). Heterochrony, Allometry, and Canalization in the Human Vertebral Column: Examples From Prehistoric Amerindian Populations [Doctoral dissertation, University of Massachusetts]

Clark, G., Panjabi, M., & Wetzel, F. (1985). Can Infant Malnutrition Cause Adult Vertebral Stenosis?. *Spine*, 10(2), 165-170. doi:10.1097/00007632-198503000-00012

Clark, G. A., Hall, N. R., Aldwin, C. M., Harris, J. M., Borkan, G. A., & Srinivasan, M. (1988). Measures of Poor Early Growth Are Correlated with Lower Adult Levels of Thymosin-α_1: Results From the Normative Aging Study. *Human Biology*, *60*(3), 435–451.

Clark, G., Hall, N., Armelagos, G., Borkan, G., Panjabi, M., & Wetzel, F. (1986). Poor growth prior to early childhood: Decreased health and life-span in the adult. *American Journal Of Physical Anthropology*, 70(2), 145-160. doi:10.1002/ajpa.1330700202

Colombo, A., Stephens, N.B., Tsegai, Z.J., Bettuzzi, M., Morigi, M.P., Belcastro, M.G., Hublin, J.J. (2019). Trabecular analysis of the distal radial metaphysis during the acquisition of crawling and bipedal walking: A preliminary study. *Bull. Mem. Soc. Anthropol*. Paris 31, 43e51.

Conceição, E., & Cardoso, H. F. V. (2011). Environmental effects on skeletal versus dental development II: Further testing of a basic assumption in human osteological research. *American Journal of Physical Anthropology*, 144(3), 463–470. doi:10. 1002/ajpa.21433

Corron, L., Wolfe, C., & Stull, K. (2021). Vertebral Neural Canal Dimensions in Contemporary Subadult Samples: Indicators of Stress, Population Variation, or Both? Presentation at the 73rd Annual Conference of the American Academy of Forensic Sciences. Virtual.

Corron, L., Wolfe, C., & Stull, K. (2023). A multifaceted exploration of ontogenetic variation in vertebral neural canal size across contemporary populations. *American Journal of Biological Anthropology*, 180, 328-351. doi:10.1002/ajpa.24675

Corsini, C. (1991). Breastfeeding, Fertility and Infant Mortality: Lessons from the Archives of the Florence Spedale degli Innocenti. In S. Matthews Grieco, C. Corsini (Eds.), Historical Perspectives on Breastfeeding (pp.63-87). Firenze, STAMPA.

Cuestas, E., Hillman, M., Galetto, S. *et al.* (2023). Inflammation induces stunting by lowering bone mass via GH/IGF-1 inhibition in very preterm infants. *Pediatr Res* 94, 1136–1144. doi:10.1038/s41390-023-02559-5

Cummins, A., & Thompson, F. (1997). Postnatal changes in mucosal immune response: A physiological perspective of breast feeding and weaning. *Immunology and Cell Biology*, 75, 419-429.

Cunningham, C., Scheuer, L., & Black, S. (2017). Developmental Juvenile Osteology (2nd ed.). San Diego, CA: Academic Press

D'Ambrosio, V, Vena, F, Marchetti, C, et al. (2019). Midtrimester isolated short femur and perinatal outcomes: A systematic review and meta-analysis. *Acta Obstet Gynecol Scand*. 98: 11–17. doi:10.1111/aogs.13470

Dalla-Zuanna, G., Rosina, A. (2011) An Analysis of Extremely High Nineteenth-Century Winter Neonatal Mortality in a Local Context of Northeastern Italy. *Eur J Population* 27, 33–55. doi:10.1007/s10680-010-9219-5

Dancause, K.N., Cao, X.J., Veru, F., Xu, S., Long, H., Yu, C., Laplante, D.P., Walker, C.D. and King, S. (2012), Brief communication: Prenatal and early postnatal stress exposure influences long bone length in adult rat offspring. *Am. J. Phys. Anthropol*., 149: 307-311. doi:10.1002/ajpa.22117

De Luca, F. (2006). Impaired Growth Plate Chondrogenesis in Children with Chronic Illnesses. *Pediatr Res* 59, 625–629. doi:10.1203/01.pdr.0000214966.60416.1b

De Onis, M., Borghi, E., Arimond, M., Webb, P., Croft, T., Saha, K., . . . Flores-Ayala, R. (2018). Prevalence thresholds for wasting, overweight and stunting in children under 5 years. *Public Health Nutrition*, 22(1), 175-179. doi:10.1017/S1368980018002434

Del Panta, L., & Pozzi, L. (2011). Age and cause mortality structure in the Italian regions at the beginning of the health transition. *Statistica*, 71(1), 23–49. doi:org/10.6092/issn.1973-2201/3603

Demirjian, A., Goldstein, H., & Tanner, J. (1973). A new system of dental age assessment. *Human Biology*, 45, 211–227.

DeWitte, SN (2018). Stress, sex, and plague: Patterns of developmental stress and survival in pre- and post-Black Death London. *Am J Hum Biol*. 30:e23073. doi:10.1002/ajhb.23073

Dimeglio, A. (1992). Growth of the Spine Before Age 5 Years. *Journal Of Pediatric Orthopaedics*, 1(2), 102-107. doi:10.1097/01202412-199201020-00003

Drusiani, R., Leoni, G., Demaria, D., & Lembo, N. (2010). Water supply of Bologna (Italy) by Roman aqueduct: History, morphology and hydraulic, from ancient time to nowadays. *Water Science and Technology: Water Supply*, 10(4), 554–560. doi.org/10.2166/ws.2010.107

Edler, R. (1977). Dental and skeletal ages in hypopituitary patients. *Journal of Dental Research*, 56(10), 1145–1153. doi: 10.1177/00220345770560100301

Elamin, F., Hector, M., & Liversidge, H. M. (2016). The timing of mandibular tooth formation in two African groups. *Annals of Human Biology*, 44, 1–29. doi:10.1080/03014460. 2016.1213313

Esan, T. A., & Schepartz, L. A. (2019). Does nutrition have an effect on the timing of tooth formation? *American Journal of Physical Anthropology*, 171, 1–11. doi:10.1002/ajpa.23987

Evans, S., Repasky, E. & Fisher, D. (2015). Fever and the thermal regulation of immunity: the immune system feels the heat. *Nat Rev Immunol* 15, 335–349. doi:10.1038/nri3843

Eveleth, P. B., & Tanner, J. M. (1990). *Worldwide Variation in Human Growth*. Cambridge University Press.

Faggioli, A. (2006). Environment and health in the history of Bologna. ARPA Rivista, 4, 14–16.

Farnum, C.E., Lee, A.O., O'hara, K., & Wilsman, N.J. (2003). Effect of Short-Term Fasting on Bone Elongation Rates: An Analysis of Catch-up Growth in Young Male Rats. *Pediatric Research*, 53(1), 33-41.

Figus, C., Stephens, N. B., Sorrentino, R., Bortolini, E., Arrighi, S., Lugli, F., Marciani, G., Oxilia, G., Romandini, M., Silvestrini, S., Baruffaldi, F., Belcastro, M. G., Bernardini, F., Erjavec, I., Festa, A., Hajdu, T., Mateovics-László, O., Novak, M., Pap, I., … Benazzi, S. (2022). Human talar ontogeny: Insights from morphological and trabecular changes during postnatal growth. *American Journal of Biological Anthropology*, 179(2), 211–228. doi:10.1002/ajpa.24596

Fornasin, A. (1998). Emigrazioni e mestieri in Carnia: la cesura del XIX secolo (pp. 19–40). CXVI: Alto.

Friebe-Hoffmann, U., Dobravsky, L., Friedl, T.W.P. (2022). The femur too short? 1373 fetuses with short femur during second-trimester screening. *Arch Gynecol Obstet* 306, 1037–1044. doi:10.1007/s00404-021-06394-z

Frisancho, A.R. (2007), Relative leg length as a biological marker to trace the developmental history of individuals and populations: Growth delay and increased body fat. *Am. J. Hum. Biol*., 19: 703-710. doi:10.1002/ajhb.20676

Frisancho, R.A., Garn, S.M., Ascoli, W. (1970a). Unequal influence of low dietary intake on skeletal maturation during childhood and adolescence. *Am. J. Clin. Nutr*. 23, 1220–1227.

Garn, S., Lewis, A., & Blizzard, R. (1965). Endocrine factors in dental development. *Journal of Dental Research*, 44(1), 243–258. doi:10.1177/00220345650440012001

Garn, S., Lewis, A., & Polachek, D. (1959). Variability of tooth formation. *Journal of Dental Research*, 38(1), 135–148. doi:10.1177/00220345590380010601

Giannelli, L. (2008). Certosa di Bologna - Storia e Memoria di Bologna.

Ginnaio, M. (2011). Pellagra in Late Nineteenth Century Italy: Effects of a Deficiency Disease. *Population (English Edition)*, *66*(3), 583. doi:10.3917/pope.1103.0583

Goetzinger, Katherine R.; Cahill, Alison G.; Macones, George A.; and Odibo, Anthony O. (2012). Isolated short femur length on second-trimester sonography: A marker for fetal growth restriction and other adverse perinatal outcomes. *Journal of Ultrasound in Medicine*. 31,12. 1935-1941.

Gooderham, E, Matias, A, Liberato, M, et al. (2019). Linear and appositional growth in children as indicators of social and economic change during the Medieval Islamic to Christian transition in Santarém, Portugal. *Int J Osteoarchaeol*, 29: 736–746. doi:10.1002/oa.2784

Goodman, A.H., & Rose, J. C. (1990). Assessment of systemic physiological perturbations from dental enamel hypoplasias and associated histological structures. *American Journal of Physical Anthropology*, *33*(S11), 59–110. doi:10.1002/ajpa.1330330506

Goodman, A.H, Leatherman, TL. (1998). Traversing the Chasm between Biology and Culture: An Introduction. In: Goodman AH, Leatherman TL, editors, *Building a New Biocultural Synthesis: Political-Economic Perspectives on Human Biology.* Ann Arbor: The University of Michigan Press, p. 3-42.

Gowland, R. L. (2015). Entangled lives: Implications of the developmental origins of health and disease hypothesis for bioarchaeology and the life course: DOHaD, Bioarchaeology, and The Life Course. *American Journal of Physical Anthropology*, *158*(4), 530–540. doi:10.1002/ajpa.22820

Griffith, J., Huang, J., Sheung-Wai, L., Xiao, F., Chi Shun Leung, J., Wang, D., & Shi, L. (2016). Population reference range for developmental lumbar spinal canal size. *Quantitative Imaging in Medicine and Surgery*, 6(6), 671-679. doi:10.21037/qims.2016.12.17

Gustafson, G., & Koch, G. (1974). Age estimation up to 16 years of age based on dental development. *Odontologisk Revy*, *25*(3), 297–306.

Halcrow, S. E., & Tayles, N. (2008). The Bioarchaeological Investigation of Childhood and Social Age: Problems and Prospects. *Journal of Archaeological Method and Theory*, *15*(2), 190–215. doi:10.1007/s10816-008-9052-x

Hall, B. K. (1999). Evolutionary developmental biology. Springer Science & Business Media.

Haro Durand, L., Mesones, R., Nielsen, F., & Gorustovich, A. (2010). Histomorphometric and microchemical characterization of maturing dental enamel in rats fed a boron-deficient diet. *Biological Trace Element Research*, 135(1–3), 242–252. doi:10.1007/s12011-009-8512-9

Harrison GA. 1992. Human genetics and variation. In: Harrison GA, Tanner JM, Pilbeam DR, Baker PT, editors. Human biology. Oxford: Oxford University Press. p 145–336.

Hermanussen, M., Largo, R. H., & Molinari, L. (2001). Canalisation in human growth: a widely accepted concept reconsidered. *European journal of pediatrics*, *160*(3), 163–167. doi:10.1007/s004310000706

Higgins, R.W. and Ruff, C.B. (2011), The effects of distal limb segment shortening on locomotor efficiency in sloped terrain: Implications for Neandertal locomotor behavior. *Am. J. Phys. Anthropol*., 146: 336-345. doi:10.1002/ajpa.21575

Hillson, S. (1996). *Dental Anthropology*. Cambridge University Press.

Hillson, S. (2014). *Tooth Development in Human Evolution and Bioarchaeology*. Cambridge: Cambridge University Press. doi:10.1017/CBO9780511894916

Hinck, V., Clark, W., & Hopkins, C. (1966). Normal Interpediculate Distances (Minimum And Maximum) In Children And Adults. *American Journal Of Roentgenology*, 97(1), 141-153. doi: 10.2214/ajr.97.1.141

Hinck, V., Hopkins, C., & Clark, W. (1965). Sagittal Diameter of the Lumbar Spinal Canal in Children and Adults. *Radiology*, 85(5), 929-937. doi: 10.1148/85.5.929

Hinck, V., Hopkins, C., & Savara, B. (1962). Sagittal Diameter of the Cervical Spinal Canal in Children. *Radiology*, 79(1), 97-108. doi: 10.1148/79.1.97

Hodson, C. M. and Gowland, R. L. (2019). Like mother, like child: investigating perinatal and maternal health stress in post-medieval London. in *The mother-infant nexus in*

anthropology: small beginnings, significant outcomes., pp. 39-64. Bioarchaeology and social theory.

Hogan, D., & Kertzer, D. (1987). The social bases of declining infant mortality: Lessons from a nineteenth-century Italian town. *European Journal of Population*, 2(3–4), 361–385. doi:10.1007/bf01796597

Holliday TM, Ruff CB. 2001. Relative variation in the human proximal and distal limb segments. *Am J Phys Anthropol* 116:26–34.

Holliday, T.W., 1999. Brachial and crural indices of European Late Upper Paleolithic and Mesolithic humans. *J. Hum.* Evol. 36, 549–566. doi:10.1016/j. jhevol.1998.0289.

Humphrey, L. T. (1998). Growth patterns in the modern human skeleton. *American Journal of Physical Anthropology*, *105*(1), 57–72. doi:10.1002/(SICI)1096-8644(199801)105:1<57::AID-AJPA6>3.0.CO;2-A

Inglis, R. M., & Halcrow, S. E. (2018). The bioarchaeology of childhood: Theoretical development in the field. In P. Beauchesne & S. C. Agarwal (Eds.), *Children and childhood in the past* (pp. 33–60). Gainesville: University Press of Florida.

Inzaghi, E., Pampanini, V., Deodati, A., Cianfarani, S. (2022). The Effects of Nutrition on Linear Growth. *Nutrients*,14,1752. doi:10.3390/nu14091752

Ipsen, C. (2006). Italy in the Age of Pinocchio, 265.

Irwin R, Raehtz S, Parameswaran N, McCabe LR (2016) Intestinal inflammation without weight loss decreases bone density and growth. *Am J Physiol Regul Integr Comp Physiol*. 311 R1149-R1157. doi:10.1152/ajpregu.00051.2016

Jantz, LM, Jantz, RL. (1999). Secular changes in long bone length and pro- portion in the United States, 1800–1970. *Am J Phys Anthropol* 110:57– 67.

Jantz, RL, and Owsley, DW. (1984) Long bone growth variation among Arikara skeletal populations. *Am. J . Phys. Anthropol*. 63:13-20.

Jarvinen, E., Salazar-Ciudad, I., Birchmeier, W., Taketo, M., Jernvall, J., & Thesleff, I. (2006). Continuous tooth generation in mouse is induced by activated epithelial Wnt/β-catenin signaling. *Proceedings of the National Academy of Sciences*, 103 (49), 18627–18632. doi:10.1073/pnas.0607289103

Jeffrey, J., Campbell, D., Golden, M., Smith, F., & Porter, 4. (2003). Antenatal Factors in the Development of the Lumbar Vertebral Canal. *Spine*, 28(13), 1418-1423.

Jernvall, J., & Thesleff, I. (2000). Reiterative signaling and patterning during mammalian tooth morphogenesis. Mechanisms of Development, 92(1), 19–29. doi:10.1016/s0925-4773 (99)00322-6

Jin, S.-W., Sim, K.-B., & Kim, S.-D. (2016). Development and Growth of the Normal Cranial Vault : An Embryologic Review. *Journal of Korean Neurosurgical Society*, *59*(3), 192. doi:10.3340/jkns.2016.59.3.192

Johnston, F (1962) Growth of the long bones of infants and young children at Indian Knoll. *Am. J. Phys. Anthropol*. 20249-254.

Jungers, WL, Cole, TM, & Owsley, DW. (1988). Multivariate analysis of relative growth in the limb bones of Arikara Indians. *Growth Dev Aging* 52:103–107.

Kamp, K. A. (2001). Where Have All the Children Gone?: The Archaeology of Childhood. *Journal of Archaeological Method and Theory*, *8*(1), 1–34.

Kertzer, D. I. (1987). Childhood and Industrialization in Italy. *Anthropological Quarterly*, *60*(4), 152. doi:10.2307/3317654

Kertzer, D. I., & White, M. J. (1994). Cheating the angel-makers: surviving infant abandonment in nineteenth-century Italy. *Continuity and Change*, *9*(03), 451. doi:10.1017/S0268416000002423

Kertzer, D. I., Koball, H., & White, M. J. (1997). Growing up as an abandoned child in nineteenth-century Italy. *The History of the Family*, 2(3), 211–228. doi:10.1016/S1081-602X(97) 90013-0

Kingsbury, B. F. (1924). The significance of the so-called law of cephalocaudal differential growth. *Anatom. Rec*., xxvii, 305-321.

Knopik, V., Maccani, M., Francazio, S., & McGeary, J. (2012). The epigenetics of maternal cigarette smoking during pregnancy and effects on child development. *Development and Psychopathology, 24*(4), 1377-1390. doi:10.1017/S0954579412000776

Kraus, B.S. (1959). Calcification of the human deciduous teeth. *Journal of the American Dental Association* 59: 1128–1136.

Kvaal, S. I., & Haugen, M. (2017). Comparisons between skeletal and dental age assessment in unaccompanied asylum seeking children. *The Journal of forensic odontostomatology*, *35*(2), 109–116.

Labrom, R. D. (2007). Growth and maturation of the spine from birth to adolescence. *The Journal of bone and joint surgery. American volume*, *89 Suppl 1*, 3–7. doi:10.2106/JBJS.F.01236

Lampl, M. (2018). Implications of Growth as a Time-Specific Event. *Nestle Nutrition Institute workshop series*, *89*, 1–11. doi:10.1159/000486488

Lampl, M. (2022). Chapter 18 - Biological models of human growth. In N. Cameron & L.M. Schell (Eds.), *Human Growth and Development (Third Edition)* (pp. 491-516,). Academic Press. doi:10.1016/B978-0-12-822652-0.00002-X

Lampl, M. & Jeanty, P. (2004), Exposure to maternal diabetes is associated with altered fetal growth patterns: A hypothesis regarding metabolic allocation to growth under hyperglycemic-hypoxemic conditions. *Am. J. Hum. Biol*., 16: 237-263. doi:10.1002/ajhb.20015

Lampl, M., Kuzawa, C.W. and Jeanty, P. (2003), Prenatal smoke exposure alters growth in limb proportions and head shape in the midgestation human fetus. *Am. J. Hum. Biol*., 15: 533-546. doi:10.1002/ajhb.10140

Lampl, M., & Schoen, M. (2017). How long bones grow children: Mechanistic paths to variation in human height growth. *American Journal of Human Biology*, *29*(2), e22983. doi:10.1002/ajhb.22983

Larsen, C. S. (2015). Bioarchaeology: Interpreting behavior from the human skeleton (2nd ed.). Cambridge: Cambridge University Press.

Leatherman, T. J., & Goodman, A. H. (2022). Critical biocultural approaches to health and illness. In M. Singer, P. I. Erickson, & C. E. Abadía-Barrero (Eds.), A Companion to Medical Anthropology, Second Edition. (pp. 26-48). Wiley Blackwell.

Leger, J., Levy-Marchal, C., Bloch, J., Pinet, A., Chevenne, D., Porquet, D., Collin, D., & Czernichow, P. (1997). Reduced Final Height and Indications for Insulin Resistance in 20 Year Olds Born Small for Gestational Age: Regional Cohort Study. *BMJ: British Medical Journal*, *315*(7104), 341–347.

Leung, S. S., Lau, J. T., Xu, Y. Y., Tse, L. Y., Huen, K. F., Wong, G. W., Law, W. Y., Yeung, V. T., Yeung, W. K., & Leung, N. K. (1996). Secular changes in standing height, sitting height and sexual maturation of Chinese--the Hong Kong Growth Study, 1993. *Annals of human biology*, *23*(4), 297–306. doi:10.1080/03014469600004532

Lewis, A., & Garn, S. (1960). The relationship between tooth formation and other maturational factors. *The Angle Orthodontist*, 30 (2), 70–77.

Lewis, M., Shapland, F., Watts, R. (2016). On the threshold of adulthood: a new approach for the use of maturation indicators to assess puberty in adolescents from medieval England. *Am J Hum Biol*;28:48–56.

Li, J., Parada, C., & Chai, Y. (2017). Cellular and molecular mechanisms of tooth root development. *The Company of Biologists*, 144, 374–384. doi:10.1242/dev.137216

Li, J., Xu, H., Shen, M., Li, S, Wang, L, Lu, Y, Li, Q. (2022)., Etiologies and Adverse Outcomes of Fetuses with Short Femur Length Based on Proportion and Percentile Categorization. *Advanced Ultrasound in Diagnosis and Therapy*, 6(1): 7-13.

Li, L., Dangour, A. D., & Power, C. (2007). Early life influences on adult leg and trunk length in the 1958 British birth cohort. *American journal of human biology: the official journal of the Human Biology Council*, *19*(6), 836–843. doi:10.1002/ajhb.20649

Lillehammer, G. (1989). A child is born. The child's world in an archaeological perspective. *Norwegian Archaeological Review*, *22*(2), 89–105. doi:10.1080/00293652.1989.9965496

Limeback, H., Schlumbohm, C., Sen, A., & Nikiforuk, G. (1992). The effects of hypocalcemia/hypophosphatemia on porcine bone and dental hard tissues in an inherited form of type 1 pseudo-vitamin D deficiency rickets. *Journal of Dental Research*, 71(2), 346–352. doi:10.1177/ 00220345920710020101

Liversidge, H. (1994). Accuracy of age estimation from developing teeth of a population of known age (0-5.4 years). *International Journal of Osteoarchaeology*, 4(1), 37–45. doi:10. 1002/oa.1390040107

Liversidge, H. (2000). Crown formation times of human permanent anterior teeth. *Archives of Oral Biology*, *45*(9), 713–721. doi:10.1016/S0003-9969(00)00055-8

Liversidge, H. (2009). Permanent tooth formation as a method of estimating age. *Frontiers of Oral Biology*, 13, 153–157. doi:10.1159/000242409

Liversidge, H. M., & Molleson, T. I. (1999). Developing permanent tooth length as an estimate of age. *Journal of Forensic Sciences*, *44*(5), 917–920.

Liversidge, H. M., Speechly, T., & Hector, M. P. (1999). Dental maturation in British children: Are Demirjian's standards applicable. *International Journal of Pediatric Dentistry*, 9, 263–269.

Livi Bacci, M. (1986). Social-group forerunners of fertility control in Europe. In: Coale, A.J. and Watkins, S.C. (eds.). *The decline of fertility in Europe*. Princeton: Princeton University Press.

Livi Bacci, M. (1990). Population and Nutrition: An Essay on European Demographic History. Cambridge University Press.

Long, F., & Ornitz, D. M. (2013). Development of the endochondral skeleton. *Cold Spring Harbor perspectives in biology*, *5*(1), a008334. doi:10.1101/cshperspect.a008334

LoPresto, S. (2020). Vertebral Neural Canal Growth and Developmental Stress: A Case Study from the American Southwest [Doctoral dissertation, George Mason University]

Louryan, S., Vanmuylder, N., & Bruneau, M. (2011). Embryology of the cervical spine and cranio-vertebral junction. In B. George, M. Bruneau, & R.F. Sptezler (Eds.), Pathology and Surgery Around the Vertebral Artery (pp. 25-28). Springer-Verlag France. ISBN 978-2-287-89786-3

Lucy, D. (2005). Introduction to statistics for forensic scientists. Wiley.

Maat GJ, Matricali B, Van Meerten EL (1996) Postnatal development and structure of the neurocentral junction. *Spine* 21:661–666

Maber, M., Liversidge, H. M., & Hector, M. P. (2006). Accuracy of age estimation of radiographic methods using developing teeth. *Forensic Science International*, 159(Suppl. 1), S68–S73.

Mailath-Pokorny M, Polterauer S, Worda K, Springer S, Bettelheim D (2015) Isolated Short Fetal Femur Length in the Second Trimester and the Association with Adverse Perinatal Outcome: Experiences from a Tertiary Referral Center. *PLoS ONE* 10(6): e0128820. doi:10.1371/journal. pone.0128820

Maresh MM. (1943). Growth of major long bones in healthy children. A preliminary report on successive roentgenograms of the extremities from early infancy to twelve years of age. *American Journal of Diseases of Children* 66:227–257.

Maresh MM. (1955). Linear growth of long bones of extremities from infancy through adolescence. *American Journal of Diseases of Children* 89:725–742.

Maresh MM. (1970). Measurements from roentgenograms. In: McCannon RW (Ed), Human growth and development. Charles C Thomas: Springfield. 157–188.

Marino, R., Tanganelli, V., Pietrobelli, A., & Belcastro, M. G. (2021). Evaluation of the auricular surface method for subadult sex estimation on Italian modern (19th to 20th century) identified skeletal collections. *American journal of physical anthropology*, *174*(4), 792–803. doi:10.1002/ajpa.24146

Mariotti, V., Zuppello, M., Pedrosi, M. E., Bettuzzi, M., Brancaccio, R., Peccenini, E., … Belcastro, M. G. (2015). Skeletal evidence of tuberculosis in a modern identified human skeletal collection (Certosa cemetery, Bologna, Italy): Skeletal Evidence of Tuberculosis. *American Journal of Physical Anthropology*, *157*(3), 389–401. doi:10.1002/ajpa.22727

Martorell, R. (1989). Body size, adaptation and function. *Human Organization* (48), 15-20.

Martorelli, R. (2011). Certosa di Bologna - Storia e Memoria di Bologna. Chiostro Annesso al Maggiore.

Massler, M., & Schour, I. (1946). Growth of the child and the calcification pattern of the teeth. *American Journal of Orthodontics and Oral Surgery*, *32*(9), 495–517. doi:10.1016/0096-6347(46)90067-1

Mathiesen, J. M., Aksglaede, L., Skibsted, L., Petersen, O. B., Tabor, A., & Danish Fetal Medicine Study Group (2014). Outcome of fetuses with short femur length detected at second-trimester anomaly scan: a national survey. *Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology*, *44*(2), 160–165. doi:10.1002/uog.13286

May, R., Goodman, A., & Meindl, R. (1993). Response of bone and enamel formation to nutritional supplementation and morbidity among malnourished Guatemalan children. *American Journal of Physical Anthropology*, 92(1), 37–51. doi:10. 1002/ajpa.1330920104

Mays, S. (1999). Linear and appositional long bone growth in earlier human populations: a case study from Mediaeval England. In Human Growth in the Past: Studies Using Bones

and Teeth, Hoppa RD, Fitzgerald CM (eds). Cambridge University Press: Cambridge; 290–312.

Mays, S., Gowland, R., Halcrow, S., & Murphy, E. (2017). Child Bioarchaeology: Perspectives on the Past 10 Years. *Childhood in the Past*, *10*(1), 38–56. doi:10.1080/17585716.2017.1301066

McDade, TW. (2003). Life history and the immune system: Steps toward a human ecological immunology. *Yrbk Phys Anthropol*, 46, 100–125.

McDonald, C. M., Olofin, I., Flaxman, S., Fawzi, W. W., Spiegelman, D., Caulfield, L. E., Black, R. E., Ezzati, M., Danaei, G., & Nutrition Impact Model Study (2013). The effect of multiple anthropometric deficits on child mortality: meta-analysis of individual data in 10 prospective studies from developing countries. *The American journal of clinical nutrition*, 97(4), 896–901. doi:10.3945/ajcn.112.047639

McPherson C. B. (2021). Examining developmental plasticity in the skeletal system through a sensitive developmental windows framework. *American journal of physical anthropology*, *176*(2), 163–178. doi:10.1002/ajpa.24338

Meadows L, Jantz R. 1995. Allometric secular change in the long bones from the 1800s to the present. *J Forensic Sci* 40:762–767.

Mehta, L., & Singh, H.M. (1972). Determination of Crown-Rump Length from Fetal Long Bones: Humerus and Femur. *Am. J. Phys. Anthropol*., 36: 165-168.

Mensforth, R.P. (1985). Relative tibia long bone growth in the Libben and Bt-5 prehistoric skeletal populations. *Am. J. Phys. Anthropol*., 68: 247-262. doi:10.1002/ajpa.1330680212

Milella, M, Franklin, D, Belcastro, MG, Cardini, A. (2021). Sexual differences in human cranial morphology: Is one sex more variable or one region more dimorphic? *Anat Rec*, 304: 2789–2810. doi:10.1002/ar.24626

Millward, D. (2017). Nutrition, infection and stunting: The roles of deficiencies of individual nutrients and foods, and of inflammation, as determinants of reduced linear growth of children. *Nutrition Research Reviews, 30*(1), 50-72. doi:10.1017/S0954422416000238

Moorrees, C. F. A., Fanning, E. A., & Hunt, E. E. (1963a). Age variation of formation stages for ten permanent teeth. *Journal of Dental Research*, 42(6), 1490–1502. doi:10.1177/00220345630420062701

Moorrees, C. F. A., Fanning, E. A., & Hunt, E. E. (1963b). Formation and resorption of three deciduous teeth in children. *American Journal of Physical Anthropology*, 21(2), 205–213. doi:10.1002/ajpa.1330210212

Moriguchi, M., Yamada, M., Miake, Y., & Nitta, Y. (2011). Immunolocalization of the factors related to Wnt signaling pathway in developing rat molar. *Journal of Hard Tissue Biology*, 20(3), 185–194. doi:10.2485/jhtb.20.185

Mousikou, M., Kyriakou, A., Skordis, N. (2023). Stress and Growth in Children and Adolescents. *Horm Res Paediatr*; 96 (1): 25–33. doi:10.1159/000521074

Mummert, A., Schoen, M., & Lampl, M. (2018). Growth and Life Course Health Development. In N. Halfon, C. B. Forrest, R. M. Lerner, & E. M. Faustman (Eds.), *Handbook of Life Course Health Development* (pp. 405–429). Cham: Springer International Publishing. https://doi.org/10.1007/978-3-319-47143-3_17

Muthuuri, J. (2021). Association of Developmental Lumbar Spinal Canal Stenosis and Stunting. *Indian Spine Journal*, 4, 149-154. doi:10.4103/isj.isj_20_20

Nanci, A., Mocetti, P., Sakamoto, Y., Kunikata, M., Lozupone, E., & Bonucci, E. (2000). Morphological and immunocytochemical ana- lyses on the effects of diet-induced hypocalcemia on enamel maturation in the rat incisor. Journal of Histochemistry & Cytochemistry, 48(8), 1043–1057. doi:10.1177/002215540004800803

Nelson, J. S., Harrington, L, Holland, E, & Cardoso, H. F. V. (2017, October 25-28). An Examination of Long Bone Length for Age in Children from a Late 19th Century Cemetery Assemblage from Bologna, Italy [Conference presentation]. CAPA 2017 Conference, Edmonton, AB, Canada.

Nelson, J., Harrington, L., Holland, E., & Cardoso, H. (2021). Does age estimated from teeth forming in different early life periods show differential discrepancy with known age?. *American Journal Of Human Biology*, 33(2). doi:10.1002/ajhb.23577

Nelson, J., Holland, E., Harrington, L., and Cardoso, H.F.V. (2020). *Do the regions of the spinal column record stress differently? An analysis of growth stunting in the vertebral neural canal.* Program of the 89th Annual Meeting of the American Association of Physical Anthropologists. *Am J Phys Anthropol*, 171: 1-321. doi:10.1002/ajpa.24023

Newman, S. L., & Gowland, R. L. (2015). The Use of Non-Adult Vertebral Dimensions as Indicators of Growth Disruption and Non-Specific Health Stress in Skeletal Populations. *American Journal of Physical Anthropology*, 158(1), 155–164.

Newman, SL, Gowland, RL, Caffell, AC. (2019). North and south: A comprehensive analysis of non-adult growth and health in the industrial revolution (AD 18th–19th C), England. *Am J Phys Anthropol*, 169: 104–121. doi:10.1002/ajpa.23817

O'Connell, D., Ho, J., Mammoto, T., Turbe-Doan, A., O'Connell, J., & Haseley, P. et al. (2012). A Wnt-Bmp feedback circuit controls intertissue signaling dynamics in tooth organogenesis. *Science Signaling*, 5(206), ra4-ra4. doi:10.1126/scisignal.2002414

Oğuz, H., Akkuş, S., Tarhan, S., Açikgözoğlu, S., & Kerman, M. (2002). Measurement of spinal canal diameters in young subjects with lumbosacral transitional vertebra. *European Spine Journal*, 11(2), 115-118. doi:10.1007/s005860100323

Ortega, N., Behonick, D. J., & Werb, Z. (2004). Matrix remodeling during endochondral ossification. *Trends in Cell Biology*, *14*(2), 86–93. doi:10.1016/j.tcb.2003.12.003

Papp, T., Porter, R., & Aspden, R. (1994). The Growth of the Lumbar Vertebral Canal. *Spine*, 19(24), 2770-2773. doi:10.1097/00007632-199412150-00006

Pedrosi ME. 2016. Age estimation and sex determination in human skeletal remains. A test of the common methods Used in anthropology for sex determination and age estimation applied to identified human European skeletal collection (Bologna, Coimbra 19th–20th C.), (Unpublished doctoral dissertation's thesis). Alma Mater Studiorum Università di Bologna, Bologna, Italy. doi: 10.6092/unibo/amsdottorato/7488.

Pelletier, DL. (1994). The Relationship between Child Anthropometry and Mortality in Developing Countries: Implications for Policy, Programs and Future Research. *Journal of Nutrition*, 124, 2047S-2081S.

Pierro, A., Cilla, S., Maselli, G., Cucci, E., Ciuffreda, M., Sallustio, G. (2017). Sagittal Normal Limits of Lumbosacral Spine in a Large Adult Population: A Quantitative Magnetic Resonance Imaging Analysis. *Journal of Clinical Imaging Science*, 7(35), 1-7. doi:10.4103/jcis.JCIS_24_17

Pietrobelli, A.; Sorrentino, R.; Durante, S.; Marchi, D.; Benazzi, S.; Belcastro, M.G. (2022). Sexual Dimorphism in the Fibular Extremities of Italians and South Africans of Identified Modern Human Skeletal Collections: A Geometric Morphometric Approach. *Biology*,*11*,1079. doi:10.3390/biology11071079

Platt, B., & Stewart, R. (1962). Transverse trabeculae and osteoporosis in bones in experimental protein-calorie deficiency. *British Journal Of Nutrition*, 16(1), 483-495. doi:10.1079/bjn19620047

Pomeroy E, Stock JT, Stanojevic S, Miranda JJ, Cole TJ, Wells JCK. 2012. Trade-offs in relative limb length among Peruvian children: Extending the thrifty phenotype hypothesis to limb proportions. *PLoS One* 7: e51795.

Pomeroy, E., Stock, J.T., Stanojevic, S., Miranda, J.J., Cole, T.J., Wells, J.C.K. (2014). Stunting, adiposity, and the individual-level "dual burden" among urban lowland and rural highland Peruvian children. *Am. J. Hum. Biol. Off. J. Hum. Biol. Counc.*, 26, 481–490.

Ponzi, D., Flinn, M. V., Muehlenbein, M. P., & Nepomnaschy, P. (2020). Hormones and human developmental plasticity. *Molecular and Cellular Endocrinology, 505*, Article 110721. doi:10.1016/j.mce.2020.110721

Porter, R. W., & Pavitt, D. (1987a). The vertebral canal: I. Nutrition and development, an archaeological study. *Spine*, *12*(9), 901–906.

Porter, R.W., Drinkall, J.N., Porter, D.E., and Thorp, L. (1987b). The Vertebral Canal: II. Health and Academic Status, a Clinical Study. Spine, 12(9), 907–906.

Pozzi, L. (2002). The determinants of infant and childhood mortality: A complex tangle in the historical research. In: Società Italiana di Statistica (ed.). Atti della XLI Riunione Scientifica, Milano 5–7 giugno. Padova: CLEUP: 77–86.

Pinhasi, R., Shaw, P., White B., & Ogden, A.R. (2006) Morbidity, rickets and long-bone growth in post-medieval Britain—a cross-population analysis, *Annals of Human Biology*, 33:3, 372-389, doi: 10.1080/03014460600707503

Racine, H.L., Serrat, M.A. The Actions of IGF-1 in the Growth Plate and Its Role in Postnatal Bone Elongation. *Curr Osteoporos Rep* **18**, 210–227 (2020). doi:10.1007/s11914-020-00570-x

Ranjan Das, D., Singh, A., Gupta, R., and Agarwal, P. (2018). A Morphometric Study of Body and Vertebral Canal of Thoracic Vertebrae & its Clinical Significance. *International Journal of Anatomy and Research*, 6(4.1), 5735-5742. doi:10.16965/ijar.2018.328

Ravi, B., and Rampersaud, R. (2008). Clinical Magnification Error in Lateral Spinal Digital Radiographs. *Spine*, 33(10), E311-E316.

Reichmann, S., and Lewin, T. (1971). Growth processes in the lumbar neural arch. *Z Anat Entwicklungsgesch*, 133, 89-101.

Rewekant, A. (2001). Do environmental disturbances of an individual's growth and development influence the later bone involution processes? A study of two mediaeval populations. *International Journal of Osteoarchaeology*, 11(6), 433-443. doi:10.1002/oa.584

Ríos, L, Terán, JM, Varea, C, Bogin, B. (2020). Plasticity in the growth of body segments in relation to height-for-age and maternal education in Guatemala. *Am J Hum Biol*. 32:e23376. doi:10.1002/ajhb.23376

Ritzman, T. B., Baker, B. J., & Schwartz, G. T. (2008). A fine line: A comparison of methods for estimating ages of linear enamel hypoplasia formation. *American Journal of Physical Anthropology*, *135*(3), 348–361. doi:10.1002/ajpa.20750

Rossi L, Migliaccio S, Corsi A, Marzia M, Bianco P, et al. (2001). Reduced growth and skeletal changes in zinc-deficient growing rats are due to impaired growth plate activity and inanition. *J Nutr*, 131(4):1142-1146.

Ruff CB. (2003). Growth in bone strength, body size, and muscle size in a juvenile longitudinal sample. *Bone* 33:317–329.

Ruff, C. B., Carfalo, E., & Holmes, M. A. (2013). Interpreting skeletal growth in the past from a functional and physiological perspective. *American Journal of Physical Anthropology*, 150, 29–37.

Ryynänen, H., Sahlberg, C., Lukinmaa, P., & Alaluusua, S. (2014). The effect of high temperature on the development of mouse dental enamel in vitro. *Archives of Oral Biology*, 59(4), 400–406. doi:10.1016/j.archoralbio.2014.01.005

Saldua, N., Okafor, C., Harris. E., and Vaccaro, A. (2015). The Role of Spinal Fusion and the Aging Spine: Stenosis without Deformity. *Surgical Treatment Modalities*, 1, 329-335.

Salhotra, A., Shah, H.N., Levi, B. *et al.* (2020). Mechanisms of bone development and repair. *Nat Rev Mol Cell Biol* 21, 696–711. https://doi.org/10.1038/s41580-020-00279-w

Samelo, N. (2015). Aspectos relativos à tutela post-mortem da Personalidade Humana no Direito Civil: uma abordagem juscivilística do problema dos cemitérios. MA thesis. School of Law, University of Coimbra.

Sarnat, B. G., & Schour, I. (1941). Enamel Hypoplasia (Chronologic Enamel Aplasia) in Relation to Systemic Disease: A Chronologic, Morphologic and Etiologic Classification. *The*

Journal of the American Dental Association, *28*(12), 1989–2000. doi:10.14219/jada.archive.1941.0307

Saunders, S. R. (2008). Juvenile skeletons and growth-related studies. In M. A. Katzenberg & S. R. Saunders (Eds.), Biological anthropology of the human skeleton (2nd ed., pp. 117–148). Wiley-Liss.

Saunders, S., & Hoppa, R. (1993). Growth deficit in survivors and non-survivors: Biological mortality bias in subadult skeletal samples. *American Journal of Physical Anthropology*, 92, 127–151. doi:10.1002/ajpa.1330360608

Scalone, F., Agati, P., Angeli, A., and Donno, A. (2016). A Micro Analysis of the Infant Mortality Decline in an Italian Sharecropping Community from 1900 to 1939. Athens

Scalone, F., & Samoggia, A. (2018). Neonatal mortality, cold weather, and socioeconomic status in two northern Italian rural parishes, 1820–1900. *Demographic Research*, 39(18), 525-560.

Scammon, R. E. (1923). A summary of the anatomy of the infant and child, in: Pediatrics (I. A. Abt, ed.), pp. 257-444, W. B. Saunders, Philadelphia.

Schweich, M. (2000). A comparison of medieval stature and body proportions at the Chichester site. Unpublished Master's dissertation, Department of Archaeological Sciences, University of Bradford, Bradford, UK.

Seeman, E. (1997). From density to structure: Growing up and growing old on the surfaces of bone. *Journal of Bone and Mineral Research*, 12(4), 509–521.

Selye, H. (1978). The Stress of Life, Revised Edition. San Francisco: McGraw-Hill.

Šešelj, M. (2013). Relationship between dental development and skeletal growth in modern humans and its implications for interpreting ontogeny in fossil hominins. *American Journal of Physical Anthropology*, 150(1), 38–47. doi:10.1002/ ajpa.22209

Šešelj, M., Sherwood, R., & Konigsberg, L. (2019). Timing of development of the permanent mandibular dentition: New reference values from the Fels longitudinal study. *The Anatomical Record*, 302, 1733–1753. doi:10.1002/ar.24108

Shapland, F., & Lewis, ME. (2013). Brief communication: a proposed osteological method for the estimation of pubertal stage in human skeletal remains. Am J Phys Anthropol 151: pp. 302–310.

Shrestha, A., More, C., Keshwar, S., Shrestha, B., & Raut, T. (2019). Nutritional status influencing orofacial developmental anomalies. *Current Oral Health Reports*, 6(3), 169–176. doi:10.1007/s40496-019-00223-8

Sinclair DC. (1989). Human growth after birth. Oxford: Oxford University Press.

Sinclair, D., & Dangerfield, P. (1998). *Human Growth after Birth* (6 edition). Oxford; New York: Oxford University Press.

Smith, B. (1991). Standards of human tooth formation and dental age assessment. In M. Kelley & C. Larsen (Eds.), *Advances in dental anthropology*. Wiley.

Smith, P., Wax, Y., & Adler, F. (1989). Population variation in tooth, jaw, and root size: A radiographic study of two populations in a high-attrition environment. *American Journal of Physical Anthropology*, 79, 197–206.

Smith, P., Wax, Y., Adler, F., Silberman, U., & Heinic, G. (1986). Post-Pleistocene changes in tooth root and jaw relationships. *American Journal of Physical Anthropology*, 70, 339–348.

Smith, S.L. and Buschang, P.H. (2004), Variation in longitudinal diaphyseal long bone growth in children three to ten years of age. *Am. J. Hum. Biol*., 16: 648-657. doi:10.1002/ajhb.20077

Smith, S.L. and Buschang, P.H. (2005), Longitudinal models of long bone growth during adolescence. *Am J Hum* Biol 17:731–745

Sofaer Derevenski, J. (1996). Where are the children? Accessing children in the past. *Archaeological Review from Cambridge*, *13*, 7–20.

Sorrentino R, Belcastro MG, Figus C, Stephens NB, Turley K, Harcourt-Smith W, et al. (2020). Exploring sexual dimorphism of the modern human talus through geometric morphometric methods. *PLoS ONE* 15(2): e0229255. doi:10.1371/journal.pone.0229255

Spake, L., & Cardoso, H. F. V. (2018). Are we using the appropriate reference samples to develop juvenile age estimation methods based on bone size? An exploration of growth differences between average children and those who become victims of homicide. *Forensic science international*, *282*, 1–12. doi:10.1016/j.forsciint.2017.10.041

Spake L, & Cardoso, HFV. (2021). Interpolation of the Maresh diaphyseal length data for use in quantitative analyses of growth. *Int J Osteoarchaeol*. 2021; 31: 232–242. doi:10.1002/oa.2942

Stephensen, C. (1999). Burden of Infection on Growth Failure. *Journal of Nutrition*, 129: pp. 534S-538S.

Suarez-Bregua, P., Guerreiro, P.M., and Rotllant, J. (2018). Stress, Glucocorticoids and Bone: A Review From Mammals and Fish. *Front. Endocrinol*, 9(526): pp. 1-8. doi:10.3389/fendo.2018.00526

Suomalainen, M., & Thesleff, I. (2010). Patterns of Wnt pathway activity in the mouse incisor indicate absence of Wnt/β-catenin signaling in the epithelial stem cells. *Developmental Dynamics*, 239(1), 364–372. doi:10.1002/dvdy.22106

Tacar, O., Demýrant, A., Nas, K., and Altindað, O. (2003) Morphology of the Lumbar Spinal Canal in Normal Adult Turks. *Yonsei Medical Journal*. 44(4), 679-685.

Takamura, K., Ohyama, S., Yamada, T. and Ishinishi, N. (1988), Changes in body proportions of Japanese medical students between 1961 and 1986. *Am. J. Phys. Anthropol*., 77: 17-22. doi:10.1002/ajpa.1330770104

Tanner, J. (1978). *Foetus into man*. Cambridge, Mass.: Harvard University Press.

Tanner, J.M. (1962). *Growth at adolescence* (2nd ed.). Thomas: Springfield, Ill.

Tanner, J.M. (1981), Growth and Maturation during Adolescence. *Nutrition Reviews*, 39: 43-55. doi:10.1111/j.1753-4887.1981.tb06734.x

Tanner, J.M.; Hayashi, T.; Preece, M.A.; Cameron, N. (1982). Increase in length of leg relative to trunk in Japanese children and adults from 1957 to 1977: comparison with British and with Japanese Americans. *Ann. Hum. Biol*, *9*, 411-423.

Temple DH. 2008. What can stature variation reveal about environ- mental differences between prehistoric Jomon foragers? Understanding the impact of systemic stress on developmental stability. *Am J Hum Biol* 20:431–439.

Temple, DH. (2014). Plasticity and constraint in response to early-life stressors among late/final Jomon period foragers from Japan: Evidence for life history trade-offs from incremental microstructures of enamel. *Am J Phys Anthropol*, *155*, 537–545.

Temple, DH. Bioarchaeological evidence for adaptive plasticity and constraint: Exploring life-history trade-offs in the human past. *Evol Anthropol*. 2019; 28: 34–46. doi:10.1002/evan.21754

Temple, DH., Lieverse, A., Bazaliiskii, V., Faccia, K., and Weber, A. (2021). Skeletal and dental maturation relative to tooth formation in prehistoric hunter-gatherers from Cis-Baikal, Siberia. Archaeological Research in Asia, 100239, 1-10. doi:10.1016/j.ara.2020.100239

Thurstans, S., Sessions, N., Dolan, C., Sadler, K., Cichon, B., Isanaka, S., Roberfroid, D., Stobaugh, H., Webb, P., & Khara, T. (2022). The relationship between wasting and stunting in young children: A systematic review. *Maternal & Child Nutrition*, 18:e13246. doi:10.1111/mcn.13246

Tirapegui, J., Ribeiro, S. M. L., Pires, I. S. de O., & Rogero, M. M. (2012). Effects of Two Different Levels of Dietary Protein on Body Composition and Protein Nutritional Status of Growing Rats. *Nutrients*, *4*(9), 1328–1337. doi:10.3390/nu4091328

Tsukasaki, M., Takayanagi, H. (2019). Osteoimmunology: evolving concepts in bone–immune interactions in health and disease. *Nat Rev Immunol*, 19, 626–642. doi:10.1038/s41577-019-0178-8

Tulsi, R. S. (1971). Growth of the human vertebral column. An osteological study. *Acta Anatomica*, 79(4), 570–580.

Tummers, M., & Thesleff, I. (2009). The importance of signal pathway modulation in all aspects of tooth development. Journal of Experimental Zoology Part B: Molecular and Developmental Evolution, 312B(4), 309–319. doi:10.1002/jez.b.21280

Tung, K., Fujita, H., Yamashita, Y., & Takagi, Y. (2006). Effect of turpentine-induced fever during the enamel formation of rat incisor. *Archives of Oral Biology*, 51(6), 464–470. doi:10.1016/j.archoralbio.2005.12.001

Tuttle, R. J. (2015). Water systems in renaissance Bologna. In The Neptune fountain in Bologna: Bronze, marble, and water in the making of a Papal City (pp. 261–274). Brepols NV.

Ubelaker, D. H. (1987). Estimating age at death from immature human skeletons: an overview. *Journal of Forensic Sciences*, *32*(5), 1254–1263.

Ubelaker, D. H., Khosrowshahi, H. (2019). Estimation of Age in Forensic Anthropology: Historical Perspective and Recent Methodological Advances, *Forensic Sciences Research*, 4(1), 1–9, doi:10.1080/20961790.2018.1549711

Ulijaszek, S. (1998). Long-term consequences of early environ- mental influences. In: Ulijaszek S, Johnston FE, Preece MA. editors. The Cambridge Encyclopaedia of Human Growth and Development. Cambridge: Cambridge University Press. p 417–421.

Ulijaszek, S. J., & Kerr, D. A. (1999). Anthropometric measurement error and the assessment of nutritional status. *British Journal of Nutrition*, 82, 165–177.

Ursu, T., Porter, R., & Navaratnam, V. (1996). Development of the Lumbar and Sacral Vertebral Canal in Utero. *Spine*, 21(23), 2705-2708. doi:10.1097/00007632-199612010-00001

van Roy, P., Barbaix, E., Clarijs, J., & Mense, S. (2001). Anatomical background of low back pain: variability and degeneration of the lumbar spinal canal and intervertebral disc. *Der Schmerz*, 15(6), 418-424. doi:10.1007/s004820100026

Verbiest, H. (1980). Stenosis of the lumbar vertebral canal and sciatica. *Neurosurgical Review*, 3(1), 75-89. doi:10.1007/bf01644422

Vercellotti, G., Stout, S. D., Boano, R., & Sciulli, P. W. (2011). Intrapopulation variation in stature and body proportions: Social status and sex differences in an Italian medieval population (Trino Vercellese, VC). *American Journal of Physical Anthropology*, 145, 203–214.

Viciano, J, Tanga, C, D'Anastasio, R, Belcastro, MG, Capasso, L. (2021). Sex estimation by odontometrics of nonadult human remains from a contemporary Italian sample. *Am J Phys Anthropol*. 2021; 175: 59–80. doi:10.1002/ajpa.24132

Vidor, G. M. (2012). *Biografia di un cimitero italiano: la Certosa di Bologna*. Il Mulino.

Waddington, C. H. (1942). Canalization of development and the inheritance of acquired characters. *Nature*, 150, 563–565. doi:10.1038/1831654a0

Wadsworth, MEJ., Hardy, RJ., Paul, AA., Marshall, SF., Cole, TJ. (2002).Leg and trunk length at 43 years in relation to childhood health, diet and family circumstances; evidence from the 1946 national birth cohort, *International Journal of Epidemiology*, 31(2), 383–390. doi:10.1093/intjepid/31.2.383

Waldman, S. (2022). Chapter 14 - Declan Mulroney: A 48-Year-Old Male With Lower Extremity Cramping and Weakness With Ambulation. In S. Waldman (Ed.), The Spine (pp.208-222). Elsevier. doi:10.1016/B978-0-323-75636-5.00014-2

Watts, R. (2011). Non-specific indicators of stress and their association with age at death in Medieval York: Using stature and vertebral neural canal size to examine the effects of stress occurring during different periods of development. *International Journal of Osteoarchaeology*, 21(5), 568-576. doi:10.1002/oa.1158

Watts, R. (2013a). Childhood development and adult longevity in an archaeological population from Barton-upon-Humber, Lincolnshire, England. *International Journal of Paleopatholog*y, 3(2), 95-104. doi:10.1016/j.ijpp.2013.05.001

Watts, R. (2013b). Lumbar vertebral canal size in adults and children: Observations from a skeletal sample from London, England. *HOMO*, 64(2), 120-128. doi:10.1016/j.jchb.2013.01.002

Watts, R. (2015). The long-term impact of developmental stress. Evidence from later medieval and post-medieval London (AD1117-1853). *American Journal Of Physical Anthropology*, 158(4), 569-580. doi:10.1002/ajpa.22810

White, T., Black, M., and Folkens, P. (2011). Human Osteology, Academic Press.

Whitehead, R. (1985). The human weaning process. *Pediatrics*, 75(1), 189-93.

WHO Expert Committee on Physical Status. (1995). Physical status: The use and interpretation of anthropometry: Report of a WHO Expert Committee. WHO Technical Report Series No., 854.

Williams, Sade, Alkhatib, Bashar, and Serra, Rosa (2019). Development of the axial skeleton and intervertebral disc. *Current topics in developmental biology* 133, 49–90. doi:10.1016/bs.ctdb.2018.11.018.

Wit, J. M., & Boersma, B. (2002). Catch-up growth: definition, mechanisms, and models. *Journal of pediatric endocrinology & metabolism : JPEM*, *15 Suppl 5*, 1229–1241.

Wong, S. C., Dobie, R., Altowati, M. A., Werther, G. A., Farquharson, C., Ahmed, S. F. (2016). Growth and the Growth Hormone-Insulin Like Growth Factor 1 Axis in Children With Chronic Inflammation: Current Evidence, Gaps in Knowledge, and Future Directions, *Endocrine Reviews*, 37 (1), 62–110, doi:10.1210/er.2015-1026

Wood, J. W., Milner, G. R., Harpending, H. C., Weiss, K. M., Cohen, M. N., Eisenberg, L. E., ... Wilkinson, R. G. (1992). The Osteological Paradox: Problems of Inferring Prehistoric Health from Skeletal Samples [and Comments and Reply]. *Current Anthropology*, *33*(4), 343–370.

Yahya, Z. A. H., & Millward, D. Joe (1994). Dietary Protein and the Regulation of Long-Bone and Muscle Growth in the Rat. *Clin Sci (Lond)*, 87 (2): 213–224. doi:10.1042/cs0870213

Yamamoto T, Oida S, Inage T. (2006). Gene Expression and Localization of Insulin-like Growth Factors and Their Receptors throughout Amelogenesis in Rat Incisors. *Journal of Histochemistry & Cytochemistry*, 54(2):243-252. doi:10.1369/jhc.5A6821.2005

Yang, Y., Li, Z., Chen, G., Li, J., Li, H., Yu, M., Zhang, W., Guo, W., & Tian, W. (2018). GSK3β regulates ameloblast differentiation via Wnt and TGF-β pathways. *Journal of Cellular Physiology*, 233(7), 5322–5333. doi:10.1002/jcp.26344

Yuii, K., Suzuki, M. And Kurachi, M. (2007), Stress Sensitization in Schizophrenia. *Annals of the New York Academy of Sciences*, 1113: 276-290. doi:10.1196/annals.1391.013

Zalel, Y., Lehavi, O., Schiff, E., Shalmon, B., Cohen, S., Schulman, A. and Achiron, R. (2002), Shortened fetal long bones: a possible *in utero* manifestation of placental function. *Prenat. Diagn*., 22: 553-557. doi:10.1002/pd.364

Zecchi, G. (1828). *Descrizione della Certosa di Bologna ora cimitero communale*. Giovanni Zecchi Calcografo.

Appendices

Appendix A

Summary statistics of diaphyseal length (mm) for each long bone (females)									
Age	Humerus			Radius			Ulna		
(years)	n	x̄	SD	n	x̄	SD	n	x̄	SD
0	2	71.59	2.64	2	54.18	2.18	1	59.84	/
0.5	6	85.41	7.97	6	63.54	4.85	6	72.04	5.49
1	7	106.38	12.69	5	73.22	10.93	4	82.99	11.76
2	2	113.55	2.58	1	83.33	/	1	92.86	/
3	1	138.03	/	/	/	/	1	108.59	/
4	1	156.44	/	1	109.57	/	1	121.22	/
5	3	153.09	12.72	3	109.29	9.11	3	119.30	10.96
6	1	171.77	/	1	122.20	/	1	131.45	/
7	1	193.00	/	1	141.50	/	1	153.00	/
8	/	/	/	/	/	/	/	/	/
9	1	202.50	/	1	152.43	/	1	/	/
10	/	/	/	/	/	/	/	/	/
11	/	/	/	/	/	/	/	/	/
Age	Femur			Tibia			Fibula		
(years)	n	x̄	SD	n	x̄	SD	n	x̄	SD
0	2	82.47	4.63	2	70.04	4.96	2	66.42	6.16
0.5	7	109.04	13.55	6	91.74	9.47	6	87.61	9.46
1	8	131.26	13.90	8	107.24	13.46	6	101.50	15.19
2	2	145.66	16.26	2	116.53	1.10	2	114.18	0.33
3	1	174.87	/	1	141.92	/	/	/	/
4	1	206.50	/	1	164.31	/	1	164.47	/
5	4	213.88	11.25	4	171.23	8.83	4	167.67	7.75
6	1	246.00	/	1	191.43	/	1	187.71	/
7	1	274.00	/	1	219.00	/	1	220.50	/
8	/	/	/	/	/	/	/	/	/
9	1	269.00	/	1	225.50	/	1	224.50	/
10	/	/	/	/	/	/	/	/	/
11	/	/	/	/	/	/	/	/	/

Appendix B

Summary statistics of diaphyseal length (mm) for each long bone (males)									
Age	Humerus			Radius			Ulna		
(years)	n	x̄	SD	n	x̄	SD	n	x̄	SD
0	1	67.88	/	1	52.60	/	1	61.04	/
0.5	1	73.43	/	1	58.09	/	1	60.01	/
1	5	108.05	10.77	3	84.82	8.37	3	93.39	8.22
2	2	121.58	14.26	2	90.10	10.87	2	98.72	14.84
3	2	136.11	4.00	1	101.54	/	1	108.02	/
4	/		/	/	/	/	/	/	/
5	1	163.69	/	1	123.97	/	1	134.87	/
6	/	/	/	/	/	/	/	/	/
7	1	188.14	/	1	134.42	/	1	145.69	/
8	/	/	/	/	/	/	/	/	/
9	1	214.29	/	1	129.20	/	1	143.47	/
10	/	/	/	/	/	/	/	/	/
11	2	/	9.49	2	150.59	6.94	2	166.02	10.58
Age	Femur			Tibia			Fibula		
(years)	n	x̄	SD	n	x̄	SD	n	x̄	SD
0	1	79.15	/	1	66.24	/	1	64.25	/
0.5	2	100.37	23.87	2	84.29	18.48	/	/	/
1	5	133.96	13.97	5	112.28	10.75	3	116.14	11.19
2	2	154.97	26.45	2	127.38	22.92	2	120.05	21.72
3	3	172.08	3.58	2	144.49	5.37	2	138.20	2.23
4	/	/	/	/	/	/	/	/	/
5	1	226.50	/	1	186.50	/	1	183.00	/
6	/	/	/	/	/	/	/	/	/
7	1	280.50	/	1	229.50	/	1	202.00	/
8	/	/	/	/	/	/	/	/	/
9	1	241.00	/	1	196.00	/	1	192.50	/
10	/	/	/	/	/	/	/	/	/
11	2	283.50	2.83	2	233.25	12.37	2	230.00	9.19

Appendix C

Mean transverse diameter and percentile measurements of each vertebra by age.

Vertebra	Measurement	Age (Years)						
		3.0-5.9	6.0-8.9	9.0-10.9	11.0-12.9	13.0-14.9	15.0-16.9	17.0-18.9
C3	Mean (mm)	20.28	20.25	20.17	20.69	/	22.79	22.71
	Median (mm)	20.44	20.88	20.17	20.69	/	22.93	22.47
	SD	0.791	1.733	/	0.997	/	1.544	0.584
	N	6	3	1	2	0	4	3
C4	Mean (mm)	21.02	21.52	22.62	22.49	/	23.18	24.07
	Median (mm)	20.66	22.16	22.62	22.49	/	23.30	23.97
	SD	0.969	1.413	0.325	1.181	/	1.390	0.577
	N	7	3	2	2	0	4	3
C5	Mean (mm)	21.72	21.82	22.97	22.91	24.43	23.91	25.24
	Median (mm)	21.68	22.16	22.97	22.91	24.43	23.95	24.20
	SD	0.945	1.352	0.375	0.834	/	0.911	1.836
	N	7	3	2	2	1	4	3
C6	Mean (mm)	22.08	22.65	22.37	23.97	25.41	24.03	25.03
	Median (mm)	22.02	22.27	22.37	23.97	25.41	24.37	25.24
	SD	0.934	1.211	0.233	0.721	/	0.785	1.213
	N	7	3	2	2	1	4	4
C7	Mean (mm)	21.12	21.38	19.68	22.12	25.28	23.62	23.37
	Median (mm)	21.04	21.29	19.68	22.12	25.28	24.19	23.83
	SD	0.979	0.568	/	1.591	/	1.41	1.596
	N	10	4	1	2	1	4	4
T1	Mean (mm)	18.45	18.00	18.74	19.49	21.86	20.35	19.70
	Median (mm)	18.18	18.25	18.74	19.49	21.86	20.44	19.83
	SD	1.038	0.713	1.881	1.683	/	1.965	1.108
	N	11	4	2	2	1	4	4
T2	Mean (mm)	15.58	15.46	16.14	17.12	18.56	17.43	17.59
	Median (mm)	15.32	15.39	16.14	17.12	18.56	18.41	17.54
	SD	0.970	0.823	0.757	1.039	/	2.170	1.474
	N	10	4	2	2	1	4	4
T3	Mean (mm)	14.52	14.85	15.31	15.82	16.91	16.67	16.31
	Median (mm)	14.31	14.89	15.31	15.82	16.91	17.20	16.48
	SD	1.252	1.145	0.969	0.488	/	1.902	1.469
	N	10	4	2	2	1	4	4
T4	Mean (mm)	13.93	14.81	14.28	15.54	16.78	16.35	15.34
	Median (mm)	13.64	14.77	14.28	15.54	16.78	16.49	15.39
	SD	1.054	1.002	/	1.308	/	1.924	1.090
	N	10	4	1	2	1	4	4

T5	Mean (mm)	14.19	14.01	15.21	14.66	17.17	15.73	15.26
	Median (mm)	14.05	13.81	15.21	14.66	17.17	16.17	15.27
	SD	0.961	0.733	1.174	1.181	/	2.832	0.765
	N	11	4	2	2	1	4	4
T6	Mean (mm)	14.01	14.39	15.44	14.95	17.21	15.72	15.39
	Median (mm)	13.81	14.42	15.44	14.95	17.21	16.25	15.42
	SD	0.899	0.416	0.651	2.065	/	2.604	1.266
	N	11	4	2	2	1	4	4
T7	Mean (mm)	14.21	14.13	15.36	15.23	17.05	15.62	15.75
	Median (mm)	13.77	14.30	15.36	15.23	17.05	16.06	15.59
	SD	0.981	0.547	0.375	1.747	/	2.576	1.187
	N	11	3	2	2	1	4	4
T8	Mean (mm)	14.44	14.27	15.43	15.03	17.56	15.73	16.05
	Median (mm)	14.28	14.28	15.43	15.03	17.56	15.79	16.28
	SD	0.840	0.785	0.071	1.174	/	2.151	1.063
	N	10	4	2	2	1	4	4
T9	Mean (mm)	14.21	14.65	15.36	15.22	16.95	15.82	16.32
	Median (mm)	14.12	14.63	15.36	15.22	16.95	15.86	15.98
	SD	0.637	0.822	0.085	1.697	/	2.371	1.373
	N	11	4	2	2	1	4	4
T10	Mean (mm)	14.43	15.55	15.38	15.67	16.47	15.67	17.43
	Median (mm)	14.26	15.01	15.38	15.67	16.47	16.19	16.45
	SD	0.916	1.989	0.891	1.457	/	2.133	2.352
	N	11	4	2	2	1	4	4
T11	Mean (mm)	15.66	16.96	16.23	18.51	17.71	16.66	17.71
	Median (mm)	15.29	16.15	16.23	18.51	17.71	17.45	17.47
	SD	1.223	2.678	1.860	1.747	/	2.473	0.536
	N	10	4	2	2	1	4	3
T12	Mean (mm)	16.91	18.00	18.66	20.97	20.64	19.33	20.82
	Median (mm)	17.21	18.78	18.66	20.97	20.64	20.32	19.77
	SD	0.923	1.438	1.047	1.138	/	2.671	2.333
	N	9	3	2	2	1	4	3
L1	Mean (mm)	18.01	19.40	20.20	21.04	21.87	20.79	21.47
	Median (mm)	18.08	19.74	20.20	21.35	21.87	21.52	22.07
	SD	0.582	0.894	0.849	0.474	/	2.546	1.286
	N	10	4	2	2	1	4	3
L2	Mean (mm)	17.62	19.81	20.23	20.76	21.63	21.37	22.10
	Median (mm)	18.05	19.84	20.23	20.76	21.63	21.63	22.51
	SD	1.306	0.980	0.834	0.453	/	2.047	1.520
	N	8	4	2	2	1	4	4
L3	Mean (mm)	17.96	20.10	20.01	22.41	21.81	21.06	22.61
	Median (mm)	17.95	20.20	22.01	22.41	21.81	21.58	22.98

	SD	0.628	0.819	0.184	0.318	/	1.616	1.198
	N	10	4	2	2	1	4	4
L4	Mean (mm)	18.2	20.2	19.7	22.6	22.5	21.8	22.0
	Median (mm)	18.2	20.3	19.7	22.6	22.5	22.5	21.9
	SD	0.770	1.657	0.771	0.615	/	2.157	0.954
	N	11	4	2	2	1	4	3
L5	Mean (mm)	20.15	21.63	22.67	24.71	25.51	23.53	26.14
	Median (mm)	20.22	21.63	22.67	24.71	25.51	23.34	26.50
	SD	1.894	3.646	0.290	2.574	/	3.973	1.795
	N	10	4	2	2	1	4	4

Mean (mm); median (mm); SD, standard deviation, *N*, number of individuals.

Appendix D

Mean anterior-posterior diameter and percentile measurements of each vertebra by age.

Vertebra	Measurement	Age (Years)						
		3.0-5.9	6.0-8.9	9.0-10.9	11.0-12.9	13.0-14.9	15.0-16.9	17.0-18.9
C3	Mean (mm)	13.71	12.90	13.69	14.68	/	13.71	14.12
	Median (mm)	13.85	12.86	13.69	14.68	/	14.22	14.05
	SD	0.658	0.129	/	0.134	/	1.368	1.082
	N	4	3	1	2	/	3	3
C4	Mean (mm)	12.98	12.65	14.03	14.96	/	13.31	13.72
	Median (mm)	12.85	12.60	14.03	14.96	/	13.69	13.71
	SD	1.107	0.142	0.594	0.368	/	1.019	1.020
	N	4	3	2	2	1	4	3
C5	Mean (mm)	13.64	12.73	14.57	14.42	13.67	13.87	14.53
	Median (mm)	13.42	12.91	14.57	14.42	13.67	14.33	14.5
	SD	0.583	0.329	0.827	1.351	/	0.984	0.501
	N	5	3	2	2	1	4	3
C6	Mean (mm)	14.13	13.02	14.27	14.19	13.13	13.78	13.98
	Median (mm)	14.38	13.27	14.27	14.19	13.13	14.05	14.58
	SD	1.030	0.531	1.160	0.997	/	0.986	1.558
	N	5	3	2	2	1	4	4
C7	Mean (mm)	14.05	13.34	13.36	13.59	13.24	13.34	13.60
	Median (mm)	14.56	13.35	13.36	13.59	13.24	13.54	13.77
	SD	1.334	0.563	/	0.919	/	1.299	0.946
	N	7	4	1	2	1	4	4
T1	Mean (mm)	14.33	14.60	14.51	14.21	13.86	13.76	14.23
	Median (mm)	14.33	14.92	14.51	14.21	13.86	13.52	14.29
	SD	1.110	0.874	0.771	0.573	/	0.771	0.315
	N	2	4	2	2	1	4	4
T2	Mean (mm)	14.64	14.47	14.14	14.42	13.77	14.40	14.36
	Median (mm)	14.64	14.33	14.14	14.42	13.77	14.30	14.31
	SD	0.163	0.320	0.898	1.068	/	1.049	0.313
	N	2	3	2	2	1	4	4
T3	Mean (mm)	14.78	14.66	15.28	14.20	12.89	14.71	13.45
	Median (mm)	14.78	14.77	15.28	14.20	12.89	14.68	13.40
	SD	/	0.649	0.424	2.008	/	1.100	12.640
	N	1	4	2	2	1	4	4
T4	Mean (mm)	14.93	14.93	14.24	14.95	13.65	15.14	14.85
	Median (mm)	14.93	15.00	14.24	14.95	13.65	15.00	15.27
	SD	0.686	1.103	/	2.051	/	0.627	1.491
	N	2	4	1	2	1	4	4

T5	Mean (mm)	15.14	15.19	13.58	15.07	13.98	15.26	14.80
	Median (mm)	15.14	15.41	13.58	15.07	13.98	15.00	15.27
	SD	0.014	0.901	1.541	1.393	/	0.828	1.087
	N	2	4	2	2	1	4	4
T6	Mean (mm)	14.94	15.08	14.84	15.59	13.48	15.27	13.79
	Median (mm)	19.94	15.26	14.84	15.53	13.48	15.24	14.11
	SD	0.184	0.654	1.541	1.747	/	0.446	1.604
	N	2	4	2	2	1	4	4
T7	Mean (mm)	14.83	14.78	15.97	15.18	14.35	14.85	14.42
	Median (mm)	14.83	14.82	15.97	15.18	14.35	14.75	14.74
	SD	0.269	0.966	0.750	1.506	/	0.668	1.245
	N	2	3	2	2	1	4	4
T8	Mean (mm)	14.64	14.81	15.61	14.76	14.34	14.75	13.74
	Median (mm)	14.64	14.82	15.61	14.76	14.34	14.83	13.87
	SD	0.219	0.875	0.629	0.806	/	0.923	1.848
	N	2	4	2	2	1	4	4
T9	Mean (mm)	14.67	14.49	16.22	14.79	13.99	14.64	14.29
	Median (mm)	14.75	14.97	16.22	14.79	13.99	15.32	13.85
	SD	0.304	1.368	0.827	0.721	/	1.665	1.803
	N	3	4	2	2	1	4	4
T10	Mean (mm)	14.76	15.36	16.56	15.40	13.44	15.09	14.68
	Median (mm)	15.14	15.59	16.56	15.40	13.44	15.30	14.30
	SD	0.646	1.249	1.147	0.141	/	1.270	1.142
	N	5	4	2	2	1	4	4
T11	Mean (mm)	15.30	16.12	16.23	16.17	16.04	15.53	14.70
	Median (mm)	15.64	16.62	16.23	16.17	16.04	15.89	14.70
	SD	1.344	1.416	0.537	0.290	/	1.391	0.515
	N	7	4	2	2	1	4	3
T12	Mean (mm)	16.81	15.88	16.43	16.55	16.83	16.36	16.27
	Median (mm)	16.62	16.40	16.43	16.55	16.83	16.58	16.34
	SD	1.524	1.772	1.421	0.078	/	0.949	0.286
	N	7	3	2	2	1	4	3
L1	Mean (mm)	16.87	16.71	16.08	15.70	16.56	15.88	16.66
	Median (mm)	16.51	16.79	16.08	15.70	16.56	15.55	16.54
	SD	1.609	1.272	1.648	0.424	/	1.087	1.379
	N	8	4	2	2	1	4	3
L2	Mean (mm)	16.66	16.65	14.86	14.21	14.97	16.37	14.68
	Median (mm)	16.75	16.66	14.86	14.21	14.97	16.22	14.76
	SD	1.321	0.918	1.266	1.188	/	1.204	1.547
	N	8	4	2	2	1	4	4
L3	Mean (mm)	15.46	15.81	14.28	14.86	14.26	14.65	14.17
	Median (mm)	15.73	15.54	14.28	14.86	14.26	14.21	14.46

	SD	1.088	0.646	1.054	1.421	/	1.902	0.909
	N	11	4	2	2	1	4	4
L4	Mean (mm)	14.6	15.7	14.4	16.1	14.6	14.9	14.2
	Median (mm)	14.7	15.7	14.4	16.1	14.6	14.1	13.8
	SD	1.790	0.299	0.877	1.704	/	3.155	1.047
	N	11	4	2	2	1	4	3
L5	Mean (mm)	16.14	16.15	15.27	15.29	17.02	15.21	17.31
	Median (mm)	14.85	15.82	15.27	15.29	17.02	14.98	16.88
	SD	2.382	0.976	0.148	2.503	/	1.079	2.877
	N	7	4	2	2	1	4	4

Mean (mm); median (mm); SD, standard deviation, *N*, number of individuals.