Microbial bioerosion: testing a new technique to differentiate stillborn, perinatal, and neonatal infants in archaeological samples.

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Abstract

First reported by Carl Wedl in 1864, microbial bioerosion is one of the most common and destructive taphonomic processes to act on skeletal material within the archaeological record. It refers to when microbes such as fungi, cyanobacteria, and bacteria breakdown mineralised hydroxyapatite to access bone collagen. Significantly lower prevalence rates for bacterial bioerosion in the bones of human and animal neonatal infants have been linked to the widely accepted sterility of foetal gastro-intestinal tracts, with the implication that infants who lack evidence for bacterial bioerosion did not survive long enough for their gut microflora to develop after birth. It is possible then, that assessments of microbial bioerosion in archaeological infant skeletal remains could be used to differentiate stillborn infants from older post-natal infants. Accurate age-at-death estimations for these individuals are important as infant mortality is considered to be a sensitive indicator of maternal and population health. The main aim of this thesis was to test the efficacy of micro-CT imaging to assessments of microbial bioerosion in archaeological infant skeletal remains from a tropical environment. This was achieved using a sample of eight infants from ‘Atele, a Chiefdom Period burial site in the Kingdom of Tonga. The second aim of this thesis was to provide insights into the biosocial context of the ‘Atele population within the Chiefdom period of Tongan culture history. Age-at-death estimations were calculated for these infants using a combination of dental and skeletal ageing standards. Micro-CT imaging was then used, as a non-destructive alternative to traditional histological techniques, to image the internal microstructure of a bone from each infant. Next, the micro-CT images captured were assessed for microibal bioerosion using the Oxford Histological Index. And lastly, the results of this assessment were compared to the results of previous research performed on the ‘Atele skeletal collection. Of the infants assessed, all but one died within the first year of life. Three of these infants died within the first three months of life. The remaining individual was given an age-at-death of *ca*. 35.8 gestational weeks, suggesting it could have been a stillborn or premature infant. All the ‘Atele infants assessed displayed extensive evidence of bacterial bioerosion, including the possible stillborn individual discussed above. As the gastro-intestinal tract is believed to be rapidly colonised at birth, particularly through feeding, it is likely this

individual was born premature and survived long enough after birth for at least its first feed. The level of perinatal and neonatal mortality within the ‘Atele population suggests that maternal health was being negatively impacted by some factor, likely a combination of nutritional and health related stress. The results of this study show micro-CT to be a valuable, non-destructive method for the imaging of the internal microstructure of bone for assessments of microbial bioerosion, however a certain degree of skill and experience is recommended to accurately interpret the information captured. This is particularly important when applying assessment methods developed for adult lamellar bone to images of infant woven bone. This study also supports the possibility of assessments of microbial bioerosion to be used to differentiate stillborn and premature infants from older post-natal infants. It also showed interesting potential for bacterial bioerosion to be used as a breastfeeding signal when used in conjunction with other methods such as stable isotope analysis. It may be beneficial to future research to test the results of this micro-CT assessment using traditional histological techniques.

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Abbreviations

AMS – Accelerator mass spectrometry

Cal BP – Calibrated years before present

DDE – Developmental defect of enamel

ECM – Extracellular matrix

GIT – Gastro-intestinal tract

LEH – Linear enamel hypoplasia

MFD – Microscopical focal destruction

Micro-CT (μCT) – Micro-computed tomography

NCPs – Non-collagenous proteins

nm – nanometer

OHI – Oxford Histological Index

SGA – Small for gestational age

μm – Micrometer

1 Introduction

Taphonomy refers to the study of the biological, physical, and chemical processes that act on the preservation of material, including skeletal material, from the point at which it enters the archaeological record to when it is excavated (Behrensmeyer and Kidwell 1985; Pokines 2013; Schotsmans et al. 2014; Stodder 2019; Turner-Walker 2008). The study of taphonomic processes can provide valuable information about the treatment of human remains around the time of death, shedding light on socio-economic and cultural practices of past populations that dictate who, how, and where individuals are buried (Behrensmeyer and Kidwell 1985; Turner-Walker and Jans 2008). The study of taphonomic processes can also provide critical information about depositional environments, such as the soil acidity, soil hydrology, and the activity of local flora, fauna, and microbes (Behrensmeyer and Kidwell 1985; Pokines 2013; Schotsmans et al. 2014; Turner-Walker and Jans 2008).

The following chapter provides an overview of microbial bioerosion, one of the most common and destructive taphonomic processes to act on skeletal material within the archaeological record (Booth 2020; Child 1995a; Collins et al. 2002). This chapter also provides an overview of the 'Atele skeletal collection studied in this thesis, the aims and objectives of this research, the issues that need to be considered when studying archaeological human remains, and a chapter outline.

1.1 Microbial bioerosion

First reported by Carl Wedl in 1864, microbial bioerosion refers to the breakdown of mineralised hydroxyapatite by microbes for access to bone collagen (Hackett 1981). This demineralisation leaves behind a distinctive pattern of tunneling, or microscopical focal destructions (MFD), of which there are two main types. The first type of MFD is known as Wedl MFD (Hackett 1981). Named after Carl Wedl, this type of bioerosion is caused by fungi and cyanobacteria (Hackett 1981). It manifests as 5-15µm tunnels that randomly branch out from the cortical surface and Haversian canals of bone (Hackett 1981; Trueman and Martill 2002). The second type of MFD is known

as non-Wedl MFD (Hackett 1981). Caused by bacteria, non-Wedl tunnels are larger ranging from 5 - 90µm and manifest as distinct foci with rims of redeposited mineral (Hackett 1981; Jans 2008).

Human skeletal remains are most commonly affected by non-Wedl MFD (Jans et al. 2004; Trueman and Martill 2002; White and Booth 2014). Research has shown the treatment of human remains around the time of death to have a profound impact on the formation of non-Wedl MFD (Jans et al. 2002; Jans et al. 2004; Nielsen-Marsh et al. 2007; Smith et al. 2007; Smith et al. 2002). Specifically, it has been hypothesised that different forms of mortuary treatment may impact the ability of endogenous bacteria, particularly that of the gut, to invade bone during decomposition and putrefaction (Booth et al. 2016; Jans et al. 2004; White and Booth 2014). For example, human skeletal remains more commonly display evidence of bioerosion, non-Wedl MFD in particular, when compared with animal skeletal remains which more commonly display evidence of Wedl MFD (Jans et al. 2004; Trueman and Martill 2002; White and Booth 2014). This has been linked to the fact that humans are traditionally buried fully articulated in distinct burial contexts, whereas animals are often butchered shortly after death, with far more variation in methods of deposition (Jans et al. 2004). During decomposition, the failure of an individual's immune system and mucosal membranes allows anaerobic gut bacteria to invade surrounding tissues (Damann and Carter 2013; Kellerman et al. 1976; Melvin et al. 1984). The butchery of animal carcasses shortly after death effectively prevents the spread of this bacteria, likely preventing the formation of non-Wedl MFD (Jans et al. 2004; Smith et al. 2007; White and Booth 2014).

Further evidence suggesting that endogenous gut bacteria play an important role in the formation of non-Wedl MFD is the relationship observed between non-Wedl MFD and age-at-death. Significantly lower prevalence rates for non-Wedl MFD have been reported for both human and animal stillborn, perinate, and neonate infants (Booth 2016; Booth et al. 2016; Jans et al. 2002; White and Booth 2014). It is suggested that this lack of non-Wedl MFD in stillborn, perinate, and neonate infants is linked to the relative sterility of foetal gastro-intestinal tracts, which are thought to be rapidly colonised by microbes at birth (Booth 2016; Booth et al. 2016; Brooks et al. 2014;

Groer et al. 2014; Mackie et al. 1999; Penders et al. 2006; White and Booth 2014). It is possible then that infants who lack evidence for non-Wedl MFD did not survive long enough for the gut microflora to form, likely dying around the time of birth (Booth 2016; Booth et al. 2016; White and Booth 2014).

If it is true that infants who do not survive long enough for the gut microflora to form do not display evidence for bacterial bioerosion, then assessments of microbial bioerosion could be used in conjunction with ageing methods to help differentiate stillborn infants from older postnatal infants in archaeological samples (Booth 2020; Booth et al. 2016). Accurate age-at-death estimations are important for perinatal infant skeletal remains because they are considered to be some of the most sensitive indicators of maternal and population health (Goodman and Armelagos 1989; Lewis 2007). *In utero*, foetuses are fully dependent on their mothers for nutrition and protection from environmental insults (Goodman and Armelagos 1989; Lewis 2007). As a result, foetal health is indirectly influenced by maternal health, something which is determined by exogenous socio-economic, cultural, nutritional, and health related factors specific to the population the mothers come from (Lewis 2007). Even after birth, maternal health impacts infant health through the passive immunity provided by breast milk (Goodman and Armelagos 1989; Lewis 2007). Not only can infant health provide valuable information about maternal health, because post-natal infants are dependent on their relatives and wider populations to supply them with the means necessary to successfully adapt to their new environment, infant mortality can provide valuable information about the adaptive success of the populations they belonged to (Lewis 2007).

1.2 Overview of sample.

The skeletal remains studied in this project come from two burial mounds, To-At-1 and To-At-2, on the grounds of Tonga College in the ‘Atele region of Tongatapu Island. With a total of 11 infants aged from birth to around one year of age, the ‘Atele skeletal collection provides an excellent sample to test the efficacy of micro-CT imaging to assessments of microbial bioerosion in archaeological infant skeletal remains from a tropical environment. Eight of these infants had bones appropriate for

micro-CT imaging, meeting the small size requirements of the micro-CT scanner used in this project (section 4.2.3).

These burial mounds were excavated by Janet Davidson in 1964 with the aim of investigating the structure of the mounds and to test the hypothesis that white sand indicated the presence of burials (Davidson 1969). Accelerator mass spectrometry (AMS) dating of these mounds presented in Stantis et al. (2015), suggests that they were in use *ca.* 460 – 0 cal BP during the Chiefdom Period (750 to 100 cal BP), a tumultuous period of Tongan culture history. As a result of the islands within the Tongan archipelago reaching resource carrying capacity during the previous Aceramic Formative Period (1550 – 750 cal BP), a number of competing regional chiefdoms emerged, with a single dynasty, the *Tu'i Tonga*, rising to political dominance during the Chiefdom Period (Burley 1998). Competition for resources also meant that the Chiefdom Period saw an increase in warfare (Burley 1998; Burley 1994). Previous research has found significantly lower δ^{15} nitrogen values in the remains of the females and subadults of the 'Atele skeletal collection suggesting that health was negatively impacted by cultural restrictions placed on the consumption of animal protein (Stantis 2015; Stantis et al. 2015). This may have implications for maternal health, which as mentioned above, indirectly impacts infant and early childhood health (Goodman and Armelagos 1989; Lewis 2007). Assessments of microbial bioerosion in infant skeletal remains may provide a new technique to explore this issue further.

A foetal femur from the University of Otago, W.D. Trotter Anatomy Museum was also selected for analysis. This bone was included in this assessment to act as a control for a non-burial depositional context, having been part of an anatomical skeletal collection. If it is accurate that infants who do not survive long enough for the gut microflora to form do not display non-Wedl MFD, then this bone should also provide a reference for what well preserved bone should look like.

1.3 Research aims and objectives

There are two aims addressed in this research. The first aim is to test the efficacy of micro-CT imaging to assessments of microbial bioerosion in archaeological infant skeletal remains from a tropical environment, and the second was to provide insights into the biosocial context of the ‘Atele population within the Chiefdom period of Tongan culture history. This was achieved through four objectives:

1. Firstly, infants from the ‘Atele skeletal collection aged from birth to around one year of age were selected using a census compiled for (Buckley 2001). As mentioned above, a foetal femur from the University of Otago, W.D. Trotter Anatomy Museum was also selected for assessment to act as a control. New, independent age-at-death estimations for these infants were performed as part of this research using dental formation, dental eruption, and diaphyseal length ageing standards. Preference was given to diaphyseal length aging methods.
2. Secondly, micro-CT imaging was used as a non-destructive method to image the internal microstructure of an appropriate bone from each infant.
3. Thirdly, the Oxford Histological Index (OHI) was used to assess the images captured of each bone for microbial bioerosion.
4. And lastly, the results of this assessment were compared to the results of previous research performed on the ‘Atele skeletal collection, particularly that which investigates the health of this population.

1.4 Issues that need to be considered when studying archaeological human remains

In addition to the issues outlined above surrounding the use of ageing standards, there are a number of issues that need to be considered when studying archaeological skeletal samples. A number of these issues center around the representativeness of a skeletal sample to the living population it came from (Pinhasi and Bourbou 2007; Waldron 1994). For a skeletal sample to be truly representative it must be comprised of all the individuals who died during the time period being studied (Waldron 1987). There

are however, a number of factors that act to lower the representativeness of a skeletal sample. Human agency is a critical factor to the representativeness of a skeletal sample (Pinhasi and Bourbou 2007). Whether based on religious, cultural, or political ideals, human agency determines not only if someone is buried, but also when, where, and how they are buried (Henderson 1987; Schotsmans et al. 2017; Waldron 1994). For example, infant remains have a long history of under- and over-representation in mortuary samples due to differential practices such as infanticide, and the cultural segregation and exclusion of these individuals from cemetery samples (Booth 2020; Halcrow and Tayles 2011; Lewis 2019; Saunders 2008).

Human agency can also determine the quantity of skeletal material recovered for study, as not all excavation strategies are designed for the recovery of skeletal material. This is particularly an issue for the ‘Atele skeletal sample, as the excavation strategy was aimed at understanding how the mounds were constructed rather than the recovery of skeletal material (Davidson 1969). This meant that only a small portion of the skeletal remains buried in the mounds were excavated and in the case of some burials, skeletal elements were left behind in the walls of the excavation squares rather than excavated (Davidson 1969). The representativeness of the infant skeletal remains studied included in this project is also reduced as this represents a subsample of the total ‘Atele skeletal collection. In addition, the infants included in this analysis are a subsample of the total infants in the ‘Atele skeletal collection. Two infants were excluded from the analysis due to a lack of skeletal material suitable for Micro-CT imaging, and an additional infant was excluded because while it was listed on the census, it could not be located.

Another issue to be considered is the consistency of the terminology used in research focusing on subadult individuals. As discussed in Chapter 4.1. there are multiple definitions of age. There is physiological age, which refers to how far along the developmental continuum an individual is, and there is chronological age, which refers an individual’s known calendar age since birth (Halcrow and Tayles 2011; Scheuer and Black 2000). It cannot be forgotten that there are many socio-cultural factors that can determine how a population will actually define an infant, child, or adult (Halcrow and Tayles 2011; Lewis 2007). This is known as an individual’s social age, and it is

important to take this into consideration when making assumptions about past populations based of age-at-death estimations (Halcrow and Tayles 2011; Lewis 2007; Lewis 2019).

1.5 Thesis outline

Chapter two provides a literature review of human bone and the taphonomic processes that impact its preservation in the archaeological record. Chapter three describes the archaeological, cultural, and geographical context of the ‘Atele skeletal collection, as well as the previous bioarchaeological research that has been performed on the sample. The context of the foetal femur from the W.D. Trotter Anatomy Museum is also discussed in Chapter three. The methods involved in the age-at-death estimations, the micro-CT imaging, and the assessment of these remains for microbial bioerosion are outlined in Chapter four, with the results outlined in Chapter five. Chapter six provides a discussion of these results in regard to the research aims and objectives, as well as some concluding remarks.

2 Literature review

The mineralised nature of bones and teeth means that it is often all that survives of an individual once they have entered the archaeological record (White and Folkens 2005). The preservation of bone upon discovery can provide valuable information about the treatment of human remains around the time of death, while also providing valuable information about the taphonomic processes that acted on these remain while buried. The following chapter provides a review of the structure and formation of bone. This includes a discussion of the molecular, microscopic, and macroscopic organisation of bone, as well as a discussion of the modelling process. This is followed by a discussion of various taphonomic processes that impact the preservation of bone from the time it is deposited within the archaeological record to when it is excavated. The processes discussed include burial practices, thermal alteration, soil hydrology, soil acidity, and plant and animal activity. The final section of this literature review discusses a taphonomic process known as microbial bioerosion. This includes a discussion of the morphological characteristics of the various types of microbial bioerosion, the origin of the bacterial responsible for bacterial bioerosion, and the application of assessments of bacterial bioerosion to age-at-death estimations.

2.1 The structure and formation of bone

Bone is structured in several hierarchical levels designed to suit the supportive, protective, and locomotive functions of bone (Roschger et al. 2017; Steiniche and Hauge 2004; Walsh et al. 2004). At a molecular level bone has a cellular and extracellular matrix (Roschger et al. 2017). On a microscopic level the cellular and extracellular matrix are arranged into osteons to form woven and lamellar bone (Roschger et al. 2017). And lastly, at a macroscopic level lamellar bone is arranged into compact and cancellous bone to form the 206 unique bones of the human skeleton (Roschger et al. 2017). These levels are discussed in the following section.

2.1.1 The molecular level of bone

Cellular matrix

Three primary cell types work together to ensure bone formation and remodeling goes to plan: osteoblasts, osteocytes, and osteoclasts. Osteoblasts regulate the deposition of the extracellular matrix (ECM) (Walsh et al. 2004). Some osteoblasts stop regulating the deposition of ECM, becoming embedded in the ECM and differentiating into osteocytes (Fratzl-Zelman and Varga 2017; Roschger et al. 2017; Walsh et al. 2004). A dense lacunar-canalicular network allows osteocytes to communicate with other cells to help regulate matrix deposition and remodelling, while also providing osteocytes with access to blood (Fratzl-Zelman and Varga 2017; Roschger et al. 2017; Walsh et al. 2004). The cells responsible for the removal of ECM are osteoclasts (Rucci and Teti 2017). Osteoclasts are derived from the monocyte/macrophage cell line of hematopoietic stem cells (Rucci and Teti 2017). They are blood-borne and travel into bone, whereas osteoblasts and osteocytes are bone-borne (Rucci and Teti 2017). Osteoblasts, osteocytes and osteoclasts work together to create a homeostasis within bone where the deposition of ECM is equal to its resorption (Rucci and Teti 2017). When they do not work together this homeostasis disappears and bone diseases will develop (Rucci and Teti 2017).

Extracellular matrix

There are two main components of the extracellular matrix of bone. About a third is made up of organic material (Steiniche and Hauge 2004). About 90% of this organic component is found in an interconnected network of mineralised collagen fibers (Kendall et al. 2018). Type 1 collagen dominates this network, but small amounts of type III, V and IX collagen can also be found (Kendall et al. 2018; Steiniche and Hauge 2004; Walsh et al. 2004). Each collagen fiber is comprised of three polypeptide chains of amino acids glysine, proline, and lysine (Kendall et al. 2018; Whitford 2005). These chains are arranged in a triple helix formation to form tropocollagen molecules that self-aggregate into collagen fibers (Kendall et al. 2018; Whitford 2005). The remaining 10% of the organic component of bone is non-collagenous proteins (NCPs) (Walsh et al. 2004). NCPs such as alkaline phosphate, osteocalcin and osteonectin are produced by

osteoblasts, while glycoproteins and proteoglycans bind to bone while circulating in the blood stream (Steiniche and Hauge 2004). Although it is well-known that the presence of collagen provides bone tissue with the robusticity and elasticity needed to resist tension, it is less clear as to the role that NCPs play (Boskey et al. 1999; Nanci 1999; Steiniche and Hauge 2004; Walsh et al. 2004). Nanci (1999) suggested that NCPs fill spaces in the collagen network, allowing mineral deposition across the entire network. Another theory put forward by Ingram et al. (1992) suggests that NCPs may play a key role in regulating matrix mineralisation and remodeling by influencing osteoblastic and osteoclastic metabolism.

The remaining two thirds of the ECM is made up of inorganic mineralised calcium phosphate crystals (hydroxyapatite) (Roschger et al. 2017; Steiniche and Hauge 2004; White and Folkens 2005). Citrate, carbonate, magnesium, sodium and fluoride ions can also be found in hydroxyapatite as impurities (Steiniche and Hauge 2004). Hydroxyapatite crystals fill gaps in the collagen network, giving bone strength and rigidity (Kendall et al. 2018; Steiniche and Hauge 2004; White and Folkens 2005). To fit these spaces hydroxyapatite crystals are considerably smaller than collagen fibers, with lengths of 16-50nm, widths of 8-20nm, and thickness' of *ca.* 2nm (Kendall et al. 2018:23).

2.1.2 The microscopic level of bone

Woven bone

ECM can be arranged in two ways to produce two forms of microscopic bone: woven bone and lamellar bone. Bone starts out as immature woven bone, characterised by a disorganised, non-uniform distribution of collagen fibers and cells (Steiniche and Hauge 2004; Stevens and Lowe 2005; Walsh et al. 2004). Woven bone is laid down during periods of rapid bone growth, fracture healing, and when an individual is experiencing a bone disease (Steiniche and Hauge 2004; Stevens and Lowe 2005).

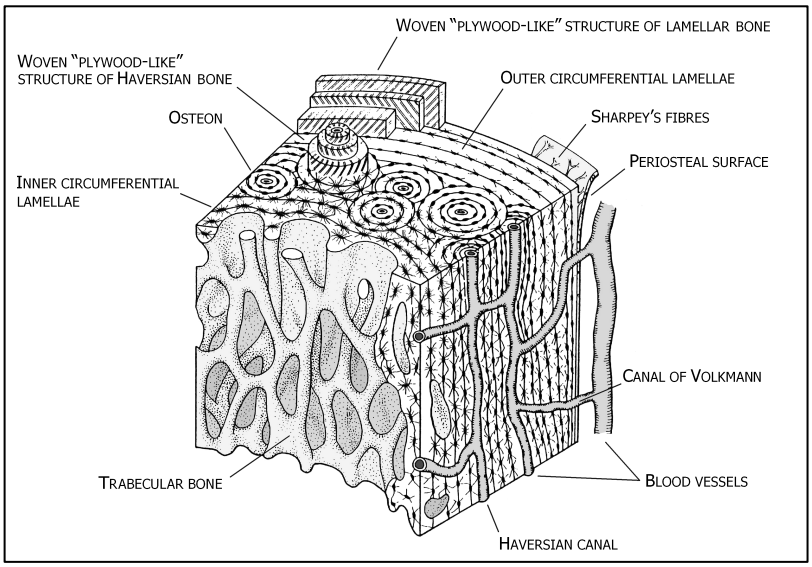

Figure 2.1. Three-dimensional schematic of the internal microstructure of compact bone (Turner-Walker 2008:9).

Lamellar bone

Gradually, woven bone is slowly replaced by lamellar bone, a more mature form of bone (Stevens and Lowe 2005; Walsh et al. 2004; White et al. 2012). Lamellar bone gets its name from its organised structure of collagen fibers arranged in parallel sheets or bundles known as lamellae (Steiniche and Hauge 2004; Stevens and Lowe 2005; White et al. 2012). Lamellae are then arranged into Haversian systems, also known as osteons (Figure 2.1) (Steiniche and Hauge 2004). Each osteon consists of four to eight concentric layers of lamellae and can measure *ca*. 300µm in diameter and *ca*. 3-5mm in length. (White et al. 2012). A Haversian canal through which blood vessels and nerve fibers pass runs through the center of each Osteon (White et al. 2012). Smaller Volkmann's canals travel both obliquely and transversely from the haversian canals transporting blood and lymph fluid (White et al. 2012). Distributed within lamellae are lacunae, small cavities that house osteocytes (White et al. 2012). Small canals known as canaliculi radiate from Haversian canals to lacunae, supplying osteocytes with nutrients (White et al. 2012). As mentioned in section 2.1.1, canaliculi also travel from lacunae to lacunae, creating a dense lacunar-canalicular network that allows osteocytes to communicate with other cells (Fratzl-Zelman and Varga 2017; Roschger et al. 2017; Walsh et al. 2004). Each osteon has a cement line boundary, a 1-5µm wide area of reduced mineralisation believed to represent the limit to which osteoblastic and

osteoclastic activity can occur (Burr et al. 1988; Steiniche and Hauge 2004; Walsh et al. 2004). Lamellar bone can also be arranged in struts. This is discussed below in regard to cancellous bone.

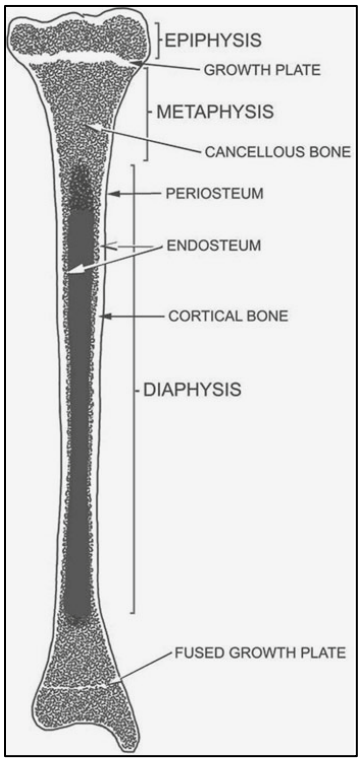

Figure 2.2. Macroscopic structure of growing bone showing primary and secondary growth centres (Steiniche and Hauge 2004:61).

2.1.3 The macroscopic level of bone

Lamellar bone exists in two forms that can be seen macroscopically: compact bone and cancellous bone. Compact bone, which is also known as cortical bone, is dense and makes up the outer layer of all bones and the shafts of long bone (Figure 2.2) (Millard 2001; Steiniche and Hauge 2004; Stevens and Lowe 2005; Walsh et al. 2004; White et al. 2012). The dense nature of compact bone combined with the hollow, tubular shape of long bones, is ideally suited to withstanding the pressures of everyday movement (Steiniche and Hauge 2004). Compact bone is replaced by cancellous bone towards the metaphyseal and epiphyseal ends of long bones (Figure 2.2) (Steiniche and Hauge 2004; Walsh et al. 2004). Also known as trabecular bone, cancellous bone is porous and lightweight with a structure similar to sponge (White et al. 2012).

Cancellous bone can also be found in the internal structure of short bones, vertebral bones and flat bones (Millard 2001; White et al. 2012). The nature of cancellous bone supports and distributes the compressive forces of everyday movement (Steiniche and Hauge 2004).

Compact bone and cancellous bone vary in the way that lamellar bone is arranged. Compact bone consists of concentric layers of lamellar bone that form osteons (Figure 2.1.) (Steiniche and Hauge 2004). Cancellous bone consists of struts of lamellar bone (Figure 2.1.) (Steiniche and Hauge 2004). These struts are known as trabeculae and on the surface of each trabeculae are lacunae that house osteocytes (Steiniche and Hauge 2004). During growth, the cavities that form between the trabeculae are filled with red bone marrow which is responsible for the production of blood cells and platelets (White et al. 2012). Over time, this red marrow is replaced with yellow marrow, a reserve for fat cells (Gurevitch et al. 2007; White et al. 2012). Yellow marrow can also be found in the medullary cavities of long bone shafts (White et al. 2012). While nutrients are supplied to compact bone through the presence of the haversian system, cancellous bone is nourished by blood vessels within the marrow cavities (White et al. 2012).

2.1.4 Bone modelling

To understand how compact and cancellous bone are arranged to form the individual bones of the human skeleton it is important to discuss how bone is modelled. Bone starts as pluripotent embryonic tissues that arise from neural crest cells and mesenchymal cells (Cunningham et al. 2016). These tissues transform into bone via two pathways: intra-membranous ossification and endochondral ossification.

During intra-membranous ossification, bone is laid down by bone-forming cells in the periosteum, the fibrous connective tissue on the surface of bone (Cunningham et al. 2016; White et al. 2012). Intra-membranous ossification results in the compact bone that covers the external surfaces of bones and the diploic bone that is found primarily in the frontal and parietal bones of the cranium (Cunningham et al. 2016; White et al. 2012).

During endochondral ossification bones start out as cartilage models (Cunningham et al. 2016; White et al. 2012). Two centres of ossification are involved: the primary and the secondary centre (Figure 2.2) (Cunningham et al. 2016; White et al. 2012). Primary ossification centres – metaphyses – control width-wise growth (White et al. 2012). In these centres bone begins as a hyaline cartilage model that becomes surrounded by periosteum (White et al. 2012). The periosteum will then deposit layers of ECM to form the shafts of long and short bones (White et al. 2012). Secondary ossification centres – epiphyses – control lengthwise growth. During growth, a cartilaginous layer known as an epiphyseal plate can be found between a bones metaphyses and epiphyses (White et al. 2012). During growth, new cartilage is laid down on the epiphyseal side of this plate by chondrocyte cells, while osteoblasts on the metaphyseal side replace old cartilage with ECM (White et al. 2012). This moves the epiphyseal plate away from the bone shaft producing lengthwise growth (White et al. 2012). Eventually, the chondrocytes stop producing cartilage while osteoblasts continue to lay down new ECM (Cunningham et al. 2016; White et al. 2012). When there is no cartilage remaining, growth will cease and the epiphysis and the metaphysis will ossify and fuse together (Cunningham et al. 2016; White et al. 2012).

2.2 Taphonomy

The body's cellular and metabolic processes control the modelling and remodelling of bone in life (Kendall et al. 2018). With death, the heart stops pumping, oxygen stops circulating, and these processes begin to fail (Damann and Carter 2013; Gill-King 1997; Kendall et al. 2018). With this failure, decomposition of the skeletal soft tissues begins, exposing bone to a wide variety of processes that determine its preservation within the archaeological record (Gill-King 1997; Kendall et al. 2018). These processes can be biological, physical, and chemical (Gill-King 1997; Kendall et al. 2018). Taphonomy refers to the study of these processes and the impact they have on the survival of material from the time it enters the archaeological record to the time it is excavated (Behrensmeyer and Kidwell 1985; Pokines 2013; Schotsmans et al. 2017; Turner-Walker 2008). Taphonomic analysis can help biological anthropologists identify the peri-mortem and post-mortem processes impacting bone preservation, as well as

allowing the processes relating to human interaction with the dead to be differentiated from natural processes occurring after deposition (Behrensmeyer and Kidwell 1985; Pokines 2013; Schotsmans et al. 2017; Turner-Walker 2008). This can provide valuable information about past populations for which there is little written record. The following section provides a discussion of a number of these processes and how they influence the preservation of bone.

2.2.1 Burial practices

It is important to discuss burial practices as a taphonomic process because human agency determines not only if someone is buried, but also when, where, and how (Henderson 1987; Schotsmans et al. 2017). These are all decisions that can have an impact on the preservation of bone and the information biological anthropologists can learn from skeletal remains. Burial is important to preservation in many ways. First of all, remains are often buried at a depth where temperature and the activity of microorganisms is low, slowing down decomposition and skeletonisation (Henderson 1987; Pokines and Baker 2013). Coffin burials can also slow down decomposition and skeletonisation by protecting skeletal remains from direct contact with the taphonomic processes acting within the burial environment (Henderson 1987; Pokines and Baker 2013). However, coffin material will eventually decay, and skeletal remains lose this protection (Henderson 1987). Patterns of poor preservation, such as staining and warping, can even be seen where skeletal remains came into direct contact with coffin material (Pokines and Baker 2013).

In some cases, bodies may not be buried at all but instead left exposed. Exposure can increase the rate of soft tissue decomposition thereby increasing the vulnerability of a skeleton to the environmental processes discussed below (Henderson 1987). In particular, exposure increases the risk of animal scavenging, disarticulation, and loss of skeletal elements (Henderson 1987).

A combination of depositional techniques may also be used (Henderson 1987). Researchers refer to such cases as ‘secondary burials,’ where a series of post-mortem events occur prior to final burial (Weiss-Krejci 2001). There are a number of reasons

why a secondary burial may occur. It could be for ritualistic reasons, such as the case for the Iroquois Indians of North America who every 10 years hold a 'Festival of the Dead,' where remains that were initially exposed on a raised platform or buried below ground are exhumed, defleshed, and reburied in a pit or placed in an ossuary (Henderson 1987). Weiss-Krejci (2001) discussed a number of cultural reasons that lead to secondary burials for members of the Babenberg and Habsburg dynasties of Europe. It occurred if an individual died away from prebuilt burial locations, if temporary storage was needed while burial places were in construction, or if the risk of communicable diseases delayed body processing (Weiss-Krejci 2001). Many members of these dynasties were also exhumed and reburied in other locations if the initial burial location was deemed inappropriate, or space constraints dictated the construction of a new burial location (Weiss-Krejci 2001). Secondary burials can have a similar impact on preservation as exposure (Henderson 1987).

2.2.2 Thermal alteration

Thermal alteration, whether through human agency or as the result of natural phenomenon, can also impact preservation. As discussed in section 2.1.1, there are two main components to bone: Inorganic hydroxyapatite and an organic component that is 90% collagen (Kendall et al. 2018). Collagen has been shown to break down with the application of heat (Symes et al. 2013). When collagen breaks down it increases the size of the porosities within the collagen-hydroxyapatite matrix, leaving bone brittle and vulnerable to damage (Symes et al. 2013). One application of heat that results in the breakdown of collagen is boiling. Boiling causes collagen to become gelatinous and it is then leached out of bone (Roberts et al. 2002; Symes et al. 2013). There are two main reasons for boiling: cooking and utilitarian purposes (Symes et al. 2013; Trujillo-Mederos et al. 2012). Cooking could refer to canabalistic behavior, however, it is also a common process applied to animal bones (Symes et al. 2013; Trujillo-Mederos et al. 2012). Utilitarian reasons for boiling can involve the defleshing of human remains (Bada et al. 1989; Trujillo-Mederos et al. 2012). This can be for ritualistic reasons or for more practical reasons such as to avoid issues related to decomposition and putrefaction (Bada et al. 1989; Trujillo-Mederos et al. 2012). For example, the remains of German

Emperor Lothar I were boiled for approximately six hours to deflesh his remains and avoid decomposition and putrefaction during the 500 km journey to his final burial location (Bada et al. 1989).

2.2.3 Soil hydrology

The movement of water through burial environments has an immense impact on preservation as water plays a key role in a number of the chemical reactions that happen during decomposition (Kendall et al. 2018; Latham and Madonna 2013; Turner-Walker 2008).

There are three important water regimes to consider when discussing the interaction between bone and groundwater: diffuse, recharge, and flow regimes (Hedges and Millard 1995; Kendall et al. 2018; Turner-Walker 2008). A diffuse regime occurs in environments where there is no movement of water, such as waterlogged environments or environments where sediments such as clay restrict the movement of water (Hedges and Millard 1995; Kendall et al. 2018; Turner-Walker 2008). Preservation of skeletal material is typically good in these environments (Turner-Walker 2008).

Environments which repeatedly become saturated with water before drying out experience a recharge water regime (Hedges and Millard 1995; Kendall et al. 2018; Turner-Walker 2008). In these environments, as the soils surrounding bone dry out, water saturated in calcium and phosphate ions is drawn out of bone (Hedges and Millard 1995; Turner-Walker 2008). This leaches bone of its mineral content and increases the porosity of bone (Hedges and Millard 1995; Kendall et al. 2018; Turner-Walker 2008). Due to the increase in porosity, more water can enter bone during the next wetting cycle and more mineral will be leached out during the next drying cycle (Hedges and Millard 1995; Kendall et al. 2018; Turner-Walker 2008). Within these environments, preservation is poor (Hedges and Millard 1995; Kendall et al. 2018; Turner-Walker 2008).

Environments where water flows easily, such as those with free-draining sandy soils, experience a flow regime (Hedges and Millard 1995; Kendall et al. 2018). Like burials within recharge regimes, burials in flow regimes are susceptible to mineral

leaching (Turner-Walker 2008). As flow regime environments never experience water saturation, mineral leaching can be constant, resulting in total mineral loss and preservation so poor that sometimes all that remains of a burial is a 'soil silhouette' (Turner-Walker 2008). An example of this can be seen in the Sutton Hoo ship burial excavated in Sulfolk, England in 1939 (Bethell and Carver 1987). Despite more than 160 artefacts being found within the burial chamber, there was no evidence of skeletal remains (Bethell and Carver 1987). Chemical examination of the burial chamber however, showed an area of concentrated phosphate, a chemical signature believed by some researchers to indicate dissolved skeletal material (Bethell and Carver 1987). Further excavations found burials in the surrounding area (Bethell and Carver 1987). Although bone fragments were found in some of these burials, most skeletons had been reduced to a soil silhouettes (Bethell and Carver 1987). Bethell and Carver (1987) suggested that the free-draining sand in the soil matrix at Sutton Hoo could have been one reason for this near complete lack of preservation.

2.2.4 Soil acidity

Similarly, the acidity of the soil at Sutton Hoo (3.5 – 5 pH) could have also contributed to the degradation of these burials (Bethell and Carver 1987). This is because hydroxyapatite becomes increasingly soluble as pH drops (Kendall et al. 2018; Kibblewhite et al. 2015; Nicholson 1996; Turner-Walker 2008). Berna et al. (2004) measured the solubility of both natural and synthetic hydroxyapatite in deionized water and pH-buffered solutions and found that hydroxyapatite was best preserved within soils where pH is 8.1 or above. This is consistent with research that suggests hydroxyapatite is most stable in environments where pH is neutral to 7.8, as most living tissue exists are near neutral pH (Berna et al. 2004; Kendall et al. 2018; Pokines and Baker 2013). The dissolution of hydroxyapatite begins as pH drops below neutral and is rapid once pH reaches 6.0 (Berna et al. 2004; Kendall et al. 2018; White and Hannus 1983).

Acidic soils can also play a role in collagen loss as it can accelerate chemical hydrolysis of collagen (Collins et al. 1995; Kendall et al. 2018). As discussed in section 2.1.1, each collagen fiber consists of a tropocollagen triple helix of polypeptide chains

(Kendall et al. 2018; Whitford 2005). Between these chains are hydrogen bonds which provide strength and stability (Collins et al. 1995). During chemical hydrolysis hydrolytic cleavage of these bonds occurs resulting in the dissolution of the polypeptide chains and the breakdown of collagen fibers (Collins et al. 1995). Collagen hydrolysis has also been shown to occur in alkaline soils (Collins et al. 1995).

2.2.5 Plant activity

Plant activity within the burial environment is another taphonomic process that can impact the preservation of bone as decomposing burials represent a highly concentrated nutrient source for plants (Pokines and Baker 2013). Root activity can be particularly detrimental to preservation. Roots may grow around burials or into bones (Pokines and Baker 2013). As they grow, roots thicken and this can lead to breakage (Pokines and Baker 2013). Roots can also secrete organic acids that aid in mineral dissolution (Pokines and Baker 2013). Plant activity by itself can have an enormous impact on preservation, but it can also provide access for microbes living in the surrounding soil to move into bone (Pokines and Baker 2013). The activity of microbes can have an enormous impact on the preservation of bone. This is discussed in section 2.3.

2.2.6 Faunal activity

Decomposing burials also represent a nutrient source for local fauna. Burrowing fauna can be particularly damaging to skeletal remains (Henderson 1987; Pokines and Baker 2013). While small organisms tend to displace small skeletal elements, larger skeletal elements can be disarticulated and lost in the tunnel systems created by larger animals (Henderson 1987; Pokines and Baker 2013). The disarticulation of skeletal elements can also result from the activity of predatory and scavenging animals, as many animal feeding patterns involve the disarticulation and transportation of skeletal elements to another location for consumption (Pokines 2013). Gnawing on bone can also aid mineral dissolution by drawing phosphate, sodium, and potassium ions from hydroxyapatite (Pokines 2013). Small bones and fragments are often ingested and the exposure to gastric acids can cause macroscopic changes to the surface of bone

indicative of gastric corrosion (Pokines 2013). Like soil acidity, gastric acids promote the chemical hydrolysis of collagen (Pokines 2013).

2.3 Microbial bioerosion

Another taphonomic process is microbial bioerosion. Microbial bioerosion occurs when microbes such as fungi, bacteria and cyanobacteria break down the mineralised portion of bone to gain access to bone collagen, leaving behind a distinctive pattern of microscopic tunneling (Child 1995a; Collins et al. 2002; Hackett 1981; Jans 2008). Evidence for bioerosion was first reported by Carl Wedl in 1864, who observed tunneling that was approximately 8µm in diameter in fossil reptilian teeth (Hackett 1981; Jans 2008). When similar tunneling was observed in freshly extracted human teeth experimentally submerged in untreated well water, Wedl suggested fungi living in the local depositional environment were responsible, ingesting bone mineral and collagen for growth (Hackett 1981; Jans 2008).

Assessments of bioerosion in archaeological, historical, and experimental samples have since investigated the microbes responsible, and how depositional environments and the treatment of remains around the time of death impact the development of bioerosion (Bell 1990; Bell et al. 1991; Bell et al. 1996; Booth 2014; Booth 2016; Booth et al. 2016; Cipollaro et al. 1998; Guarino et al. 2006; Hanson and Buikstra 1987; Hollund et al. 2012; Jans et al. 2002; Jans et al. 2004; Nielsen-Marsh and Hedges 2000; Nielsen-Marsh et al. 2007; Smith et al. 2007; Smith et al. 2002; White and Booth 2014; Yoshino et al. 1991). Recent studies (Booth et al. 2016; White and Booth 2014), have also shown the potential for assessments of bioerosion to aid in age-at-death estimations, especially that of stillborn and short-lived post-natal infants, due to significantly lower prevalence rates of bioerosion observed in the bones of these individuals compared to older infants. The microflora of the gut is thought to play a significant role in the formation of bioerosion, so the lack of bioerosion in these individuals could be explained by the widely accepted sterility of foetal gastro-intestinal tracks (Booth et al. 2016; White and Booth 2014). The lack of age-at-death records for archaeological populations can make it difficult to achieve accurate age-at-death

estimations, particularly for short-lived infants as it can be difficult to differentiate stillborn infants from older post-natal infants. The use of ageing standards developed using samples that are often from different environmental, chronological, and socio-economic contexts, reduces the accuracy of age-at-death estimations. Accurate age-at-death estimations for perinatal individuals are crucial when studying archaeological human remains, as infants are an important part of a population demographic, with the ability to provide researchers with a wealth of information about population health, palaeopathology, the cultural practices and beliefs that governed the treatment of these individuals after death, and the adaptive success of past populations (Goodman and Armelagos 1989; Halcrow et al. 2017; Lewis 2007).

2.3.1 Morphological characteristics of microbial bioerosion

The first morphological descriptions of microbial bioerosion were provided by Hackett (1981), who analysed evidence of bioerosion, or what he referred to as microscopical focal destructions (MFD), in 113 human bone sections from several different environmental and chronological contexts. Hackett (1981) described two types of bioerosion: centrifugal Wedl MFD and non-Wedl MFD. These are outlined below.

Centrifugal Wedl MFD

The tunnels described by Wedl in 1864 (cited in Hackett 1981) fall into what Hackett (1981) described as centrifugal Wedl tunnelling (Wedl MFD for short). Hackett (1981) describes these tunnels as ranging from 5 – 10µm in diameter, and branching out in a random fashion from the periosteal surface and Haversian canals of bone (Figure 2.3). Trueman and Martill (2002) recognized two distinctive types of Wedl tunneling. Type one Wedl MFD are simple, randomly branching tunnels about 10 – 15µm in diameter (Figure 2.3) (Trueman and Martill 2002). These tunnels are the most common form of Wedl tunnels and are not influenced by the microstructure of bone (Trueman and Martill 2002). Type two Wedl tunnels form a more complex network of smaller tunnels approximately 5µm in diameter, that branch out from Haversian canals (Figure 2.3) (Trueman and Martill 2002). Hackett (1981) suggested that fungi are responsible for this type of bioerosion. In an attempt to identify the type of fungi responsible,

Hackett (1981) buried pieces of sterilized compact bone in room temperature garden soil for one year. Tunnelling was seen in two pieces of bone but none of the six species of fungi that were isolated from these bones produced tunnelling in further experiments (Hackett 1981). Marchiafava et al. (1974) were able to identify *Mucor frensenius* as a bone-boring fungus when fragments of human vertebrae were buried in flower pots filled with garden soil. The results of this experiment may not be applicable to studies of bioerosion in archaeological bone, as the samples were heat treated at 200º Celsius for 20 minutes prior to burial (Marchiafava et al. 1974; Turner-Walker 2008). This would have had serious implications for the collagen content of the bone, making the bone more vulnerable to any microbes present in the soil (Turner-Walker 2008). The experiments by Marchiafava et al. (1974) showed that the mineral dissolved by fungi is transported out of the bone. Why is yet to be understood (Jans 2008).

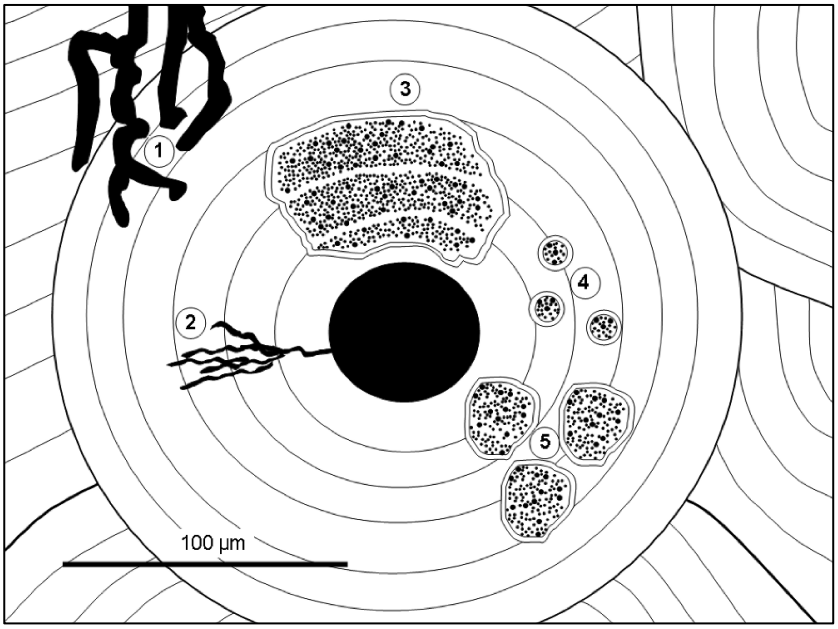

Figure 2.3. Schematic of a secondary osteon showing the different morphologies of microbial bioerosion: 1) type one Wedl MFD, 2) type two Wedl MFD, 3) Lamellate non-Wedl MFD, 4) Linear-Longitudinal non-Wedl MFD, and 5) Budded non-Wedl MFD (Jans 2008:400).

In aquatic environments, cyanobacteria have been shown to produce Wedl-like tunneling (Bell et al. 1996; Davis 1997). When examining bioerosion in a sample of archaeological and fossil bird bones, Davis (1997) observed Wedl-like tunneling that radiated parallel to the periosteal surface of bone. The same tunneling was seen when

Davis (1997) experimentally deposited modern bird bone in various freshwater and saltwater environments. Macroscopic examination of the modern bird bone found that areas of bioerosion corresponded with areas of blue-green staining from cyanobacteria (Davis 1997). Bell et al. (1996) also identified cyanobacteria as the microorganism responsible for bioerosion in human skeletal material from various Canadian environmental contexts. Light was identified as the primary influencer of the prevalence and pattern of bioerosion in both these studies, as cyanobacteria are dependent on light for their metabolism (Bell et al. 1996; Davis 1997). For this reason, only skeletal material exposed to light displays cyanobacteria bioerosion. An example of this can be seen in the skull of a double crested cormorant that Davis (1997) partially buried. Only the portion of the skull not buried was colonized and bioeroded by cyanobacteria (Davis 1997).

Non-Wedl MFD

Hackett (1981) also observed three patterns of bioerosion that were morphologically different to Wedl MFD: linear-longitudinal, budded, and lamellate tunneling (Figure 2.3). Hackett (1981) categorised these as non-Wedl MFD, and suggested that bacteria were responsible. All three patterns of bacterial bioerosion form from the Haversian canal of an osteon outwards until the cement line (the boundary between osteons) is reached (Hackett 1981; Jans 2008). Bacterial bioerosion can also be differentiated from Wedl MFD by the presence of a hypermineralised rim around the tunnels, believed to be where bacteria have redeposited dissolved mineral (Hackett 1981; Jans 2008).

Linear-longitudinal tunnels are the smallest tunnels produced by bacteria (Hackett 1981). These tunnels are generally small, with a diameter of 5 – 10μm, and circular (Hackett 1981). Budded tunnels are larger with a diameter of 10-60μm and get their name from side shoots that "bud" off at irregular intervals of about 80-90μm (Hackett 1981). Lamellate tunnels are generally a similar size as budded tunnels with a diameter of 10-60μm (Hackett 1981). Lamellate tunnels are rounded and curve to conform with the layers of lamellae in an osteon (Hackett 1981; Jans 2008).

2.3.2 Origin of the bacterial responsible for non-Wedl MFD in bone

There has been much debate as to whether the bacteria responsible for bacterial bioerosion of bone has an exogenous (external) or endogenous (internal) origin. This question is explored in the following section. To do so it is important to first discuss the mechanisms involved in microbial decomposition of bone.

Mechanisms involved in microbial decomposition of bone

As discussed in section 2.1.1, the extracellular matrix of bone is made up of an interconnected network of mineralised hydroxyapatite crystals and mineralised collagen fibers. Microbes can enter bone through any of the naturally occurring porosities in bone, but because hydroxyapatite crystals are considerably smaller than collagen fibers, the gaps in this network through which collagen can be accessed are too small for any molecule or microbe larger than water to fit through (Child 1995a; Child 1995b). As a consequence, microbes must first dissolve the mineralised hydroxyapatite component of bone using metabolically produced organic acids, causing bioerosion, before they can access and breakdown the collagen component of bone (Booth 2014; Child 1995b; Jans 2008). Under normal conditions, bone collagen with its triple helix structure is also resistant to microbial attack, requiring microbes to be capable of producing enzymes known as collagenases, that promote collagen hydrolysis, and enzymes known as proteases, which help shorten the collagen polypeptides (Child 1995a; Child 1995b). Bacteria capable of producing collagenase and protease enzymes are common and can be found both within depositional environments and within decomposing remains, the greatest abundance of which is in the gut (Booth 2014).

An exogenous model for non-Wedl MFD

With ideas influenced by the focus on fungi in the early research into bioerosion, the bacteria responsible for non-Wedl MFD were first thought to be exogenous in origin, attacking bone once skeletonisation had occurred (Booth 2020; Hackett 1981; Piepenbrink 1986). Hackett (1981) suggested that bacteria of the *Actinomyces* genus living in soil may be responsible, invading bone where fungi have already produced

Wedl tunneling, feeding on their waste products. An exogenous model for non-Wedl MFD would suggest that depositional environments, and the taphonomic processes that occur within these environments, play a central role in the development of non-Wedl MFD by controlling the activity of soil bacteria (Booth 2014).

A central argument for an exogenous model for non-Wedl MFD is that soil bacteria can only access bone late in the post-mortem period, after skeletonisation has occurred (Hackett 1981; Piepenbrink 1986). Research into the timing of the development of bioerosion varies greatly in its findings. Yoshino et al. (1991), for example, attempted to determine how long after death bioerosion developed by experimentally burying, exposing, and submerging in sea water 51 human humeral bone samples. These samples were periodically removed from their contexts, sectioned, and analysed using microradiography and scanning electron microscopy to determine whether bioerosion had occurred (Yoshino et al. 1991). Overall, evidence of non-Wedl MFD was not seen in samples earlier than five years post-mortem, at which point Yoshino et al. (1991) argues skeletonisation occurred for these samples and soil microbes would have had access to the skeleton for the first time. One buried sample showed evidence for bacterial bioerosion after 2.5 years, but this was attributed to the shallow burial of this sample having caused rapid skeletonisation (Yoshino et al. 1991). Research by Bell et al. (1996) argues that bacterial bioerosion can occur earlier than what was suggested by Yoshino et al. (1991). In this study 11 human bone samples from terrestrial, intertidal, and lacustrine contexts were examined for bioerosion (Bell et al. 1996). These samples covered a wide span of post-mortem intervals, from three months to 83 years (Bell et al. 1996). Non-Wedl MFD was seen as early as three months post-mortem in a tibial fragment collected from an exposure type deposition (Bell et al. 1996). This is considerably earlier than the five years proposed by Yoshino et al. (1991), and much earlier than the only observation of non-Wedl MFD in an exposure context reported by Yoshino et al. (1991), which was 15 years. This variation in the timing of non-Wedl MFD suggests that time does not play a critical role it its development. Bell et al. (1996) suggested that non-Wedl MFD could occur even earlier than what was observed in this project, possibly by endogenous bacteria which, although known to play a role in the decomposition and putrefaction of bodily soft tissues, had as of yet not been implicated in the development of non-Wedl MFD. The

role of endogenous bacteria in the development of non-Wedl MFD is discussed in detail below.

If soil bacteria are responsible for non-Wedl MFD, site specific patterns in histological preservation could be expected. It was noted by Bell et al. (1996), that the depositional environment of the samples they studied influenced the type of bioerosion observed. For example, a unique form of bioerosion was seen in the tooth of a drowning victim retrieved from an intertidal zone of saltwater (Bell et al. 1996). The tunneling on this tooth was characterised by peripheral tunneling around the neck of the tooth, invading the cementum and dentine (Bell et al. 1996:133). This form of tunneling was also seen in an earlier study by Bell et al. (1991), in the remains of sailors that died in the Mary Rose shipwreck (1545 A.D). The mandibles and maxillae studied by Bell et al. (1991) came from five cemetery contexts ranging from the Bronze Age to Medieval Period, and from the Mary Rose shipwreck. The mandibles and maxillae from the cemetery burials displayed characteristically bacterial bioerosion, whereas those of the Mary Rose shipwreck displayed only the unique tunneling observed by Bell et al. (1996). This pattern of bioerosion is now known to be caused by cyanobacteria (Bell et al. 1996; Davis 1997).

Nielsen-Marsh and Hedges (2000) attempted to identify the aspects of depositional environments which have the greatest impact on the histological alteration of bone. The histological preservation of 134 human and animal bones from 8 archaeological and modern sites spanning the last 12,000 years were assessed in this project (Nielsen-Marsh and Hedges 2000). Each of these sites are located within northwest Europe and have very different environmental contexts (Nielsen-Marsh and Hedges 2000). The best histological preservation was seen in samples that came from either waterlogged environments or from dry environments where there was little change in water level (Nielsen-Marsh and Hedges 2000). The lowest levels of histological preservation were seen in sample that came from environments where water levels fluctuated regularly (Nielsen-Marsh and Hedges 2000). Together this suggests that soil hydrology plays an important role in histological preservation. Despite this, Nielsen-Marsh and Hedges (2000) noted that histological preservation can vary significantly within and between sites, suggesting that while soil hydrology plays an

important role, histological preservation is impacted by the depositional environment in ways that were not clear.

A number of researchers (Jans et al. 2002; Jans et al. 2004; Nielsen-Marsh et al. 2007; Smith et al. 2007:1486; Smith et al. 2002) have contributed to a large European funded project aimed at investigating the archeological and environmental factors, particularly those related to depositional soils, that have major effects on the histological preservation of archaeological bone. A total of 261 animal and human bones from 41 European archaeological sites from a variety of environmental and chronological contexts were studied in this project. The bones studied fell into four categories of histological preservation and degradation (Nielsen-Marsh et al. 2007; Smith et al. 2007; Smith et al. 2002). Either bones were well preserved histologically, had suffered accelerated collagen hydrolysis, catastrophic mineral dissolution, or microbial attack (Nielsen-Marsh et al. 2007; Smith et al. 2007; Smith et al. 2002). The only correlation between burial environment and histological preservation was observed in bones that had suffered catastrophic mineral dissolution, the bulk of these bones coming from acidic soils (Nielsen-Marsh et al. 2007; Smith et al. 2007; Smith et al. 2002). Bones displaying bioerosion and bones that were well preserved histologically were equally represented in acidic burial environments, suggesting that a factor other than soil chemistry plays a dominant role in determining histological preservation (Nielsen-Marsh et al. 2007; Smith et al. 2007; Smith et al. 2002). It was suggested that early post-mortem taphonomic processes could be this determining factor (Jans et al. 2002; Jans et al. 2004; Nielsen-Marsh et al. 2007; Smith et al. 2007; Smith et al. 2002). For example, bone from bodies deposited articulated displayed consistently higher levels of non-Wedl MFD across the entire sample, whereas bone from bodies that had been disarticulated showed consistently lower levels of non-Wedl MFD (Jans et al. 2004). This has implications for an endogenous model for non-Wedl MFD and is discussed in the next section.

If exogenous bacteria are responsible for non-Wedl MFD then bone from sterile environments would show no evidence of non-Wedl MFD. Guarino et al. (2006) assessed the histological preservation of 27 femurs excavated from Pompeii. Good histological preservation of bone microstructure was seen in 18 of the femurs examined

(Guarino et al. 2006). The remaining nine femurs showed varying levels of histological preservation, with evidence of lamellate non-Wedl MFD (Guarino et al. 2006). This result is consistent with earlier research by Cipollaro et al. (1998), who studied the histological preservation of 13 bones excavated from the house of Caius Iulius Polybius in Pompeii. Good histological preservation was observed in eight of the bones examined, and poor preservation in the remaining five bones (Cipollaro et al. 1998). Pompeii has spent the last 2000 years buried under approximately five to six meters of pyroclastic ash from the eruption of Mount Vesuvius in 79 A.D. (Guarino et al. 2006). The extremely high temperatures of this ash meant it was sterile, likely inhibiting exogenous bacterial attack (Guarino et al. 2006). Attack by endogenous bacteria could explain why non-Wedl MFD was observed in these bones despite the sterility of the burial environment (Guarino et al. 2006). Further evidence for endogenous bacteria being responsible for this bioerosion is that in the effected samples from the house of Caius Iulius, only the periosteal surface was preserved (Cipollaro et al. 1998). Preservation of the periosteal and endosteal margins of bone is a commonly observed pattern in bone effected by non-Wedl MFD (Bell et al. 1996; Booth 2014; Booth et al. 2016; Hanson and Buikstra 1987; Hollund et al. 2012; Jans et al. 2004; White and Booth 2014). If exogenous bacteria are responsible for non-Wedl MFD, it would be expected that the formation of non-Wedl MFD follow an outwards-in trajectory (Jans et al. 2004). The consistently observed preservation of the periosteal and endosteal margins of bone, as well as non-Wedl MFD often being observed in association with Haversian canals, suggests that bacterial attack follows an inward-out trajectory (Bell et al. 1996; Booth 2014; Booth et al. 2016; Hanson and Buikstra 1987; Hollund et al. 2012; Jans et al. 2004; White and Booth 2014).

An endogenous model for non-Wedl MFD

With increasing evidence for an endogenous model for non-Wedl MFD, the focus of research has shifted to an investigation of the role of endogenous bacteria, particularly that of the gut, during decomposition and putrefaction. As mentioned in section 2.2, The modelling and remodelling of bone is controlled by the body's cellular and metabolic processes (Kendall et al. 2018). With death, the heart stops pumping,

oxygen stops circulating, and these processes begin to fail (Damann and Carter 2013; Gill-King 1997; Kendall et al. 2018). The failure of an organism's immune system and mucosal membranes during decomposition, allows anaerobic gut bacteria to invade all bodily tissues via the vascular system (Bell et al. 1996; Booth 2016; Booth et al. 2016; Child 1995a; Gill-King 1997; Janaway 1987; Trueman and Martill 2002). These bacteria can then invade and spread through bone using the Haversian systems (Child 1995a). The use of the Haversian systems for bacterial spread could explain why the formation non-Wedl MFD often follows an inward-out trajectory (Booth 2014; Hanson and Buikstra 1987; Jans et al. 2004).

A correlation between putrefaction and the prevalence of bone bioerosion has been seen when comparing human and animal remains. As part of the large EU funded project mentioned in the previous section, Jans et al. (2004) analysed bioerosion in a sample of 261 human and animal skeletal remains, 177 of which displayed evidence of bioerosion. Several differences were observed between the animal and human bones affected, the first being that significantly more human bone (75%) was affected by bioerosion than animal bone (57%) (Jans et al. 2004). These samples also differed in regard to the type of bioerosion observed, with non-Wedl MFD being observed in 74% of human bone compared to 34% of animal bone (Jans et al. 2004). Wedl MFD was less commonly observed within the human remains while it was the dominant form of attack within the animal remains (Jans et al. 2004). A relative lack of Wedl MFD was seen in an earlier study by Bell (1990), who observed Wedl MFD in only two of 76 adult femora and tibiae samples taken from archaeological and modern skeletal collections. Jans et al. (2004) suggested that although these differences could be related to the lower porosity of animal bone, they could also be related to differences in the post-mortem treatment of animals compared to that of humans (Jans et al. 2004). In archaeological sites for example, animal bones are more likely to be excavated from refuse or settlement layers, where higher levels of organic and humic material decrease the activity of bacteria (Jans et al. 2004). Jans et al. (2004) also suggested that these differences could be related to the practice of animal butchery impacting the ability of endogenous bacteria to spread throughout a carcass. Research has shown that the alimentary system of organs (the pancreas, intestines and stomach) are the first to breakdown during putrefaction, releasing the gut microflora into the surrounding hard

and soft tissues (Damann and Carter 2013; Kellerman et al. 1976; Melvin et al. 1984). As butchery results in the disarticulation of animal carcasses shortly after death, expansion of the gut microflora into surrounding elements is prevented, and as a result, bacterial attack is also prevented (Jans et al. 2004; Smith et al. 2007; White and Booth 2014). Human remains are often excavated from distinct burial contexts and often buried fully articulated, allowing the gut microflora to freely invade all skeletal elements (Jans et al. 2004; Smith et al. 2007; Trueman and Martill 2002).

Another correlation between bacterial bioerosion of bone and putrefaction is the relationship observed between the prevalence of bioerosion and age-at-death. Many researchers have reported significantly lower prevalence rates of bioerosion in the remains of both human and animal stillborn, perinatal, and neonatal infants when compared to older individuals (Booth 2016; Booth et al. 2016; Jans et al. 2002; White and Booth 2014). Jans et al. (2002:348) reported two "strikingly well preserved" infants when discussing the histological analysis of bioerosion in 16 individuals from two archaeological sites: Nijmegen in The Netherlands and Kits Corner in the United Kingdom. As the aim of this study was to discuss the importance of histological analysis to archaeological heritage management, why these infants displayed such a different level of histological preservation was not discussed. A low prevalence rate for bioerosion in infant remains was also seen in an experimental study by White and Booth (2014). In this study, White and Booth (2014) buried six pig carcasses, three belonging to juveniles and three belonging to stillborn pigs, for one year. Another three juveniles and three stillborn pig carcasses were sub-aerially exposed (White and Booth 2014). Pig carcasses are commonly used in experimental archaeology as they have a similar body weight, sub-cutaneous fat distribution, diet, and gut microbiota to humans (Booth 2020; White and Booth 2014:93). The aim of this study was to test whether patterns in the prevalence of bioerosion can be used to reconstruct taphonomic events, burial practices in particular (White and Booth 2014). Thin sections were taken from the femora of each carcass and examined for bioerosion (White and Booth 2014). The thin sections from all six stillborn carcasses, irrespective of treatment, lacked any evidence for bacterial bioerosion (White and Booth 2014). Booth (2016) also found age-at-death to be an influential factor in the prevalence of bioerosion when examining thin sections from 301 individuals from 25 European archaeological sites to investigate the relationship

between funerary treatment and bioerosion. 15 of the 31 neonatal samples displayed no evidence for non-Wedl MFD (Booth 2016). This is significantly higher than all other age groups, with only 1 out of 22 children, 4 out of 35 juveniles, and 19 out of 189 adults, lacking evidence of non-Wedl MFD (Booth 2016). These 15 neonates came from a variety of contexts where most other remains displayed extensive bioerosion (Booth 2016). It is therefore unlikely that funerary treatment played a role in this lack of non-Wedl MFD (Booth 2016).

White and Booth (2014) and Booth (2016) suggested that the relative lack of non-Wedl MFD in stillborn and short-lived infants could be explained the widely accepted sterility of foetal gastrointestinal tracts (GIT) (Brooks et al. 2014; Groer et al. 2014; Mackie et al. 1999; Penders et al. 2006). At birth, this sterility is thought to be rapidly lost with the introduction of microbes through contact with family members, medical professionals, the birthing environment, and with feeding (Bäckhed et al. 2015; Brooks et al. 2014; Ferretti et al. 2018; Groer et al. 2014; Mackie et al. 1999; Penders et al. 2006). Feeding, whether through breastfeeding or the hand-feeding of breastmilk substitutes, is believed to be particularly crucial in the colonisation and maturation of the neonate GIT (Bäckhed et al. 2015; Mueller et al. 2015). The possible sterility of the foetal GIT could explain the lack of bacterial bioerosion observed in some short-lived infants if they did not live long enough for their GIT to be colonised (Booth 2016; Booth et al. 2016; White and Booth 2014). Especially as the greatest abundance and diversity of microbial cells in the human microbiota, including collagenase and protease producing bacteria, can be found in the GIT (Booth 2016; Booth et al. 2016; Riedel et al. 2014; White and Booth 2014). If infants did not survive long enough for the gut microbiome to form postnatally, then the bacteria responsible may not have been available to produce non-Wedl MFD. Some researches however, argue that a prenatal microbiome may exist (Ardissone et al. 2014; Booth 2020; Ferretti et al. 2018; Groer et al. 2014; Jiménez et al. 2005; Jiménez et al. 2008; Matamoros et al. 2013; Mueller et al. 2015). Jiménez et al. (2005) for example, isolated bacteria in nine samples of cord blood collected from healthy neonates born by caesarean section. In a later study, Jiménez et al. (2008) isolated bacteria in samples of meconium collected from healthy neonates who had not yet been fed. In another study (Ardissone et al. 2014), bacterial RNA was amplified from samples of meconium collected from 52 infants ranging from 23 weeks

gestation to 41 weeks gestation. The results of these studies challenge the idea that foetal GIT's are sterile until birth, suggesting the presence of a prenatal microbiome. While the possibility of a prenatal microbiome is a growing field of interest, it is still widely accepted by researchers that the GIT of foetuses are sterile (Booth 2020; Brooks et al. 2014; Groer et al. 2014; Mackie et al. 1999; Matamoros et al. 2013; Mueller et al. 2015; Penders et al. 2006).

The possibility that the lack of non-Wedl MFD observed in the remains of some short-lived infants could be related to the supposed sterility of foetal GIT's was tested by Booth et al. (2016). The level of histological preservation in ten Romano-British perinates was compared to three of the adults previously studied by Booth (2016). Only one of the three adult samples lacked evidence of non-Wedl compared to half of the perinates (Booth et al. 2016). Of the ten perinates, four were aged as being preterm with the remaining six being older infants (Booth et al. 2016). Of the four preterm perinates, three lacked any evidence of non-Wedl MFD compared to two of the six older perinates (Booth et al. 2016). While these results are consistent with the results of the previously mentioned studies (Booth 2016; Jans et al. 2002; White and Booth 2014), the sample size was too small to test whether these results were statistically significant (Booth et al. 2016).

2.3.3 The use of bacterial bioerosion in age-at-death estimations

If the relative sterility of the foetal GIT does mean that only infants who survived long enough after birth for their gut microbiome to be colonised will display bioerosion, assessments of bioerosion may provide a new technique for the differentiation of stillborn infants from older post-natal infants in archaeological samples. As discussed above, in addition to making up a crucial part of a population demographic, infant skeletal remains can provide researchers with valuable information about population health, palaeopathology, the cultural practices and beliefs that governed the treatment of these individuals after death, and the adaptive success of past population (Halcrow et al. 2017; Lewis 2007). This makes accurate age-at-death estimations for these individuals essential. Observations of dental development and

measurements of diaphyseal length are the most commonly applied measures of foetal and neonatal age-at-death (Halcrow et al. 2017; Lewis 2007; Ubelaker 1989). Both these methods require a standard for comparison and a number of issues arise with the use of these standards (Halcrow et al. 2017; Lewis 2007; Ubelaker 1989). In particular, these standards are specific to the sample upon which they are based, but a number of biological and socio-economic variables can result in interpopulation differences in growth (Halcrow et al. 2017; Lewis 2007; Ubelaker 1989). For this reason, the standards used for age estimation are not always relevant to the sample they are applied to, resulting in inaccuracies in age-at-death estimations (Halcrow et al. 2017; Lewis 2007; Ubelaker 1989). The issues involved in the use of ageing methods are discussed fully in Chapter 4.1.

2.4 Conclusion

The aim of this chapter was to provide a detailed review of the structure and formation of bone and the taphonomic processes that impact its preservation within the archaeological record. A taphonomic process known as microbial bioerosion was highlighted as it is one of the most common and destructive taphonomic processes to impact the preservation of skeletal remains in the archaeological record (Child 1995a; Collins et al. 2002). Studies have shown microbial bioerosion to have interesting implications for research into burial environments, burial practices, and how the early post-mortem treatment of remains can impact bacterial decomposition of bone (Booth 2016; Jans et al. 2002; White and Booth 2014). Recently, assessments of microbial bioerosion have shown interesting implications for age-at-death estimations, as a lack of non-Wedl MFD has been observed in the skeletal remains of stillborn, perinatal, and neonatal infants (Booth 2016; Booth et al. 2016; Jans et al. 2002; White and Booth 2014). It has been suggested that this lack of non-Wedl MFD is linked to the relative sterility of the foetal gastro-intestinal tract, something which thought to be rapidly lost around birth (Booth 2016; Booth et al. 2016; Brooks et al. 2014; Groer et al. 2014; Mackie et al. 1999; Penders et al. 2006; White and Booth 2014). It is possible that infants who do not display evidence of non-Wedl MFD did not survive long enough for their gut microbiome to form (Booth 2016; Booth et al. 2016; White and Booth 2014).

If this is accurate, assessments of microbial bioerosion may provide a useful technique for the differentiation of perinatal infants from older infants and stillborn infants in archaeological samples.

3 Materials

Infant skeletal remains from two archaeological Tongan burial mounds, To-At-1 and To-At-2, were examined in this thesis. Recent dating of these mounds suggests they were in use *ca*. 460 – 0 cal BP, during the Chiefdom period of Tongan culture history (Stantis et al. 2015). These remains are housed in the University of Otago's Department of Anatomy, and permission to study them has been granted by the Kingdom of Tonga. This chapter provides the geographic, climatic, and cultural context of these mounds, as well as descriptions of the excavations of each mound and the variety of bioarchaeological research that has since been produced using the sample. A foetal femur (O2.D44) from the W.D. Trotter Anatomy Museum was also selected for this assessment as a non-burial depositional context control. This chapter concludes with a brief discussion of the provenance of this specimen.

3.1 'Atele skeletal collection

3.1.1 Geographic and climatic context

To-At-1 and To-At-2 are located in the 'Atele region of Tongatapu Island (Figure 3.2). Tongatapu Island is the largest of the more than 160 islands within the Kingdom of Tonga (Burley 1998). There are three major island groups within the Kingdom, the Tongatapu group to the south, Vava'u group to the north, and the central Ha'apai (Burley 1998) (Figure 3.1). These islands can be divided into two chains. To the west is a chain of volcanic islands and sea mounts, and to the east is a chain of non-volcanic islands dominated by small, shallow, raised coral limestone islands (Burley 1998). Although Tongatapu is part of this eastern non-volcanic chain, volcanic eruptions in the western chain have deposited layers of nutrient-rich volcanic ash across the island making it one of the most fertile islands within the kingdom (Burley 1998). A large central lagoon and an extensive network of barrier reefs would have also provided extensive shellfish and marine resources for exploitation (Burley 1998). The climate in Tonga is tropical, with a hot, wet season from December through April, and a cool, dry

season from May through November (Weather-and-Climate 2019). Temperatures range from about 17°C to as high as about 33°C, with an annual rainfall of about 1600mm (Weather-and-Climate 2019).

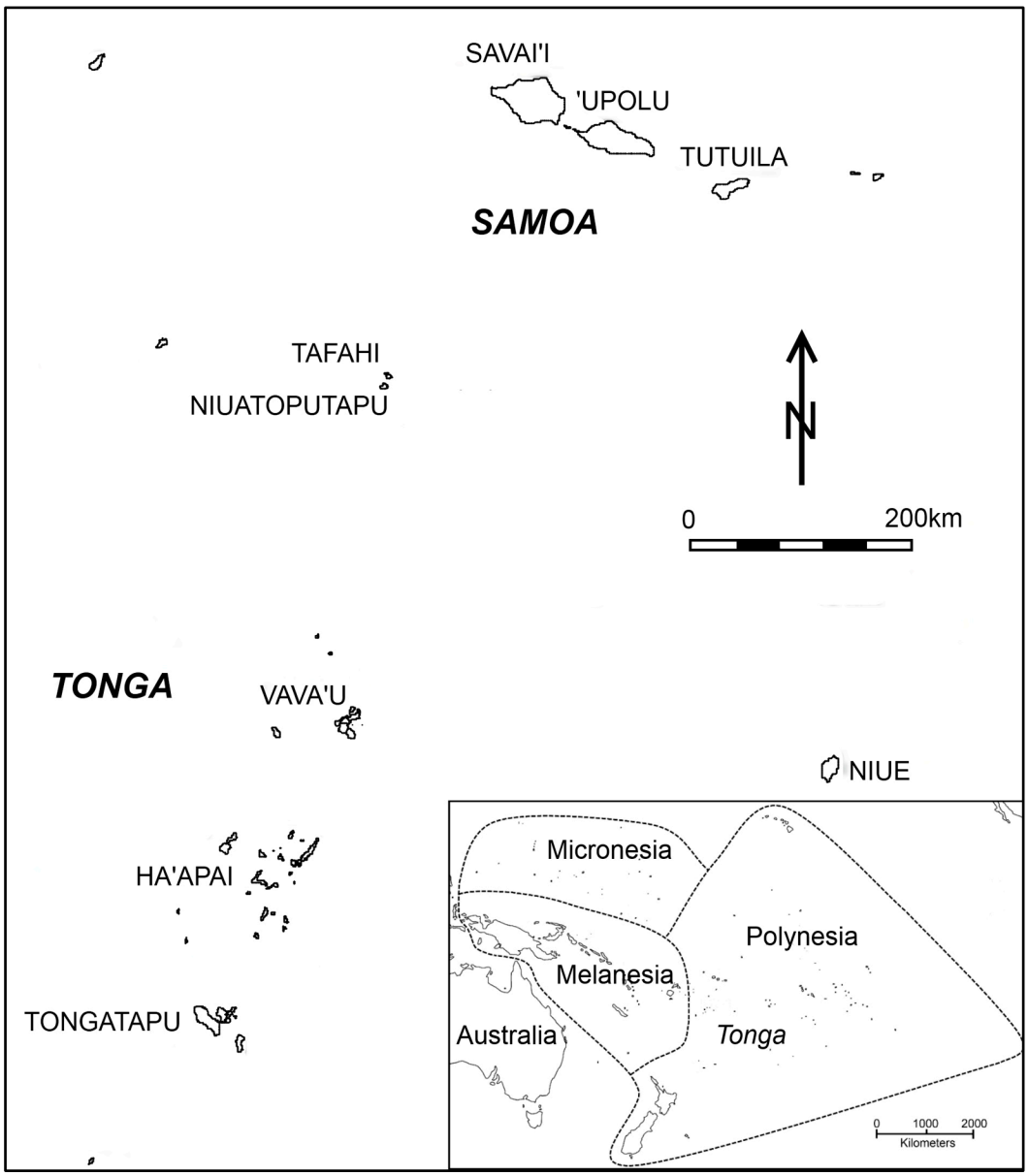

Figure 3.1. Map of the Tongan Archipelago showing the three main island groups: Tongatapu, Vava'u, and Ha'apai (Burley et al. 2015:3).

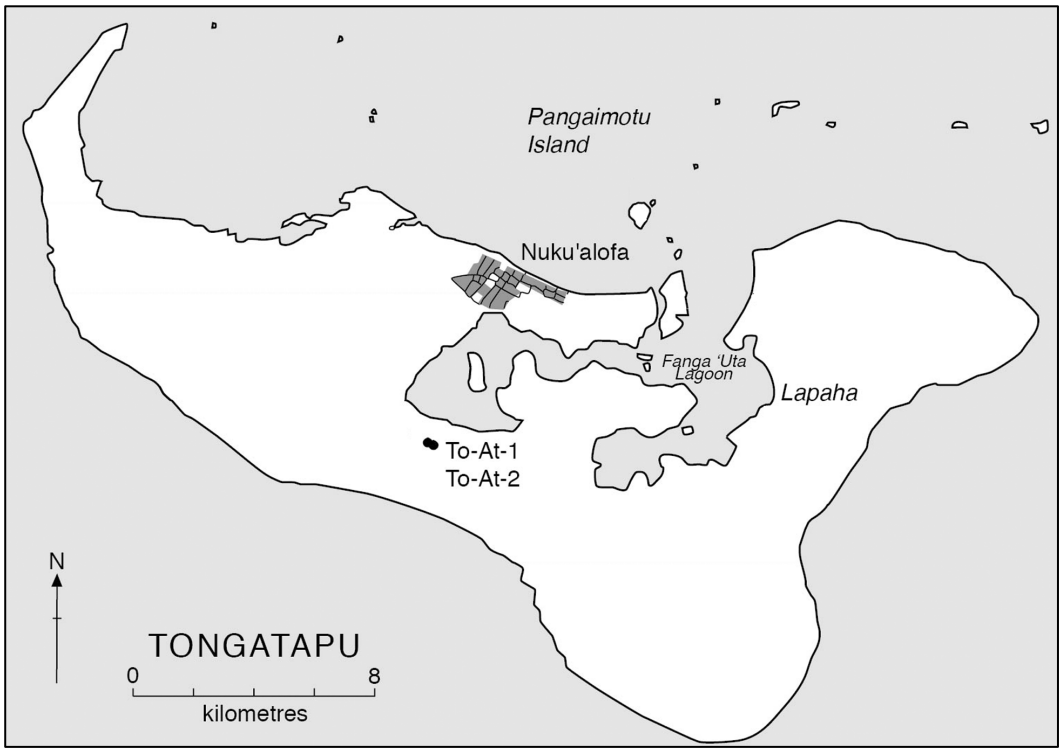

Figure 3.2. Map of Tongatapu Island showing the location of the ‘Atele burial mounds (To-At-1 and To-At-2) (Stantis et al. 2015:2).

3.1.2 Cultural context

Recent Accelerator Mass Spectrometry (AMS) dating of burials from both mounds has given an age range of *ca*. 460 – 0 cal BP, placing the use of these mounds within the Chiefdom Period of Tongan culture history (Stantis et al. 2015). The following section provides an overview of the culture history of Tonga, including the three periods that preceded the Chiefdom Period: The Eastern Early Lapita Ceramic Period, the Polynesian Plainware Ceramic Period, and the Aceramic Formative Development Period.

The Eastern Early Lapita Ceramic Period

The first people to occupy the Tongan archipelago arrived nearly 3000 years ago in an easterly migration of the Lapita cultural complex out of the Bismarck Archipelago (Burley 1998; Campbell 2001; Kirch 2017). Recent High precision Uranium/Thorium (U/Th) dating of coral files suggests Lapita people made landfall in Tonga around 2846

– 2830 cal BP at Nukulela on Tongatapu Island (Burley et al. 2012). Lapita people then rapidly moved into the rest of the archipelago, reaching the Ha'apai island group by 2772 – 2759 cal BP and the Vava'u island group by 2805 – 2760 cal BP (burley et al., 2015). These dates fall into what Burley (1998) defined as the Eastern Early Lapita Ceramic period (2850 – 2650 BP). Lapita settlements were small and coastal, allowing for easy access to the water for navigation and for the exploitation of marine resources (Burley 1998; Kirch 2017). To supplement a marine rich diet, the Lapita people brought with them a "transported landscape" of key horticultural plants and animals such as pigs, chickens, and dogs (Burley 1998; Campbell 2001; Kirch 2017). The Lapita cultural complex is also known for its ceramic series. These ceramics were produced in a range of vessel forms and decorative styles, and had both utilitarian and social functions (Kirch 2017). The most well-known marker of the Lapita cultural complex is dentate stamped ceramics, where stamps of finely carved teeth were impressed onto the surface of the ceramics (Kirch 2017).

The Polynesian Plainware Ceramic Period

Production of dentate stamped ceramics appears to have ceased around 2650 BP, marking the end of the Early Eastern Lapita Ceramic Period and the beginning of the Polynesian Plainware Ceramic Period (2650 – 1550 BP) (Burley 1998). During this period dentate stamped pottery was replaced by undecorated plainware and the range of vessel forms produced significantly decreased (Burley 1998). Subsistence also changed with the reliance on natural resources decreasing with the intensification of agriculture (Campbell 2001). Settlement patterns changed as a result, with populations growing and spreading out to cover the islands (Campbell 2001).

The Aceramic Formative Development Period

By 1550 BP ceramic production ceased entirely in Tonga, marking the beginning of the Aceramic Formative Development Period (1550 – 750 BP) (Burley 1998). The complete loss of ceramics during this period makes the identification of occupation sites difficult, with earth ovens being the most commonly identified archaeological feature from this time period (Burley 1998; Campbell 2001; Storey

2008). The difficulty in identifying sites does not mean that the Tongan population was not thriving. In fact, population levels are believed to have been steady, with resource carrying capacity likely reach during the Aceramic Formative Development Period (Green 1973; Poulsen 1974). Changes to the political and social organisation of Tongan society would have been needed to adapt to this, and these changes would have laid the foundations for the complex Chiefdom Period that followed (Burley 1998; Green 1973; Poulsen 1974).

The Chiefdom Period

This apparent 'void' in Tongan prehistory lasts until the appearance of monumental architecture during the Chiefdom Period (750 – 150 BP) (Burley 1998). Reaching resource carrying capacity during that Aceramic Formative Development Period resulted in an increase in competition for land (Burley 1998). This lead to the formation of a number of competing regional chiefdoms (Burley 1998). During the Chiefdom Period, a single dynasty, the *Tu'i Tonga*, rose to political dominance (Burley 1998). Based on Tongatapu Island, the *Tu'i Tonga* maintained control throughout the entire archipelago by employing a system of regional chiefs, or *'Eiki*, each of whom controlled an ancestral estate (Burley 1998; Kirch 1990; Kirch 2017). These chiefs ensured the transmission of prestige goods back to Tongatapu for the annual first fruits ceremony (*'inasi*) (Kirch 1990). During these ceremonies, produce and prestige goods were offered to the *Tu'i Tonga* in recognition of their political dominance (Kirch 1990). As *Tu'i Tonga* were believed to be direct descendants of the gods, this was also considered be a tribute to the god Hikuleo in exchange for successful food yields and good fortune throughout the coming year (Clark and Reepmeyer 2014).

As mentioned above, construction of monumental architecture began in the Chiefdom period (Burley 1998). This monumental architecture takes a variety of forms but is dominated by earthen or stone mounds (Burley 1994; Kirch 1990). These mounds had a variety of functions. They were used for burials, as chiefly sitting platforms, for sports such as pigeon snaring, and as foundations for buildings (Burley 1994; Kirch 1990). Conical water wells have also been observed (Burley 1994). Traditionally, these wells were a visual representation of chiefly power, used at the chiefs discretion, located

on chiefly land, and commonly found in association with chiefly burial mounds (Burley 1994).

Monumental architecture also came in the form of fortifications. The Chiefdom period saw an increase in warfare, especially between 150 – 100 BP when the political organisation collapsed and civil war broke out as chiefs began to compete for power and land (Burley 1998; Burley 1994). This had an immense impact on traditional Tongan lifeways, with a shift away from a diverse settlement pattern to nucleated communities associated with fortifications (Burley 1994). European contact during this time only added to the conflict with the introduction of cannons and firearms (Burley 1998; Burley 1994; Van Der Grijp 1993). Aided also by support from Methodist missionaries from Great Britain, one chief, Taufa'ahau, emerged as the ranking chief for all of Tonga (Van Der Grijp 1993). Taufa'ahau become the first official King of Tonga in 1845 (Van Der Grijp 1993).

3.1.3 Excavation

The 'Atele burial mounds are located on the grounds of Tonga College, a 220 acre area of land in the centre of Tongatapu Island (Figure 3.1) (Davidson 1969). These mounds were excavated by Janet Davidson in 1964. A detailed report of these excavations can be found in Davidson (1969). Prior to excavation, the grounds of the college were extensively surveyed for burial mounds (Davidson 1969). A grouping of three very large burial mounds were recorded near the entrance of the college, with an addition 11 smaller burial mounds also recorded (Davidson, 1969). Eight of these smaller mounds conformed with what McKern (1929) described as *tanuanga*, a relatively small mound constructed out of either soil or sand that was used to mark commoner burials. The other three were larger, conforming with what McKern (1929) described as *faitoka*, conical mounds with sloping sides and buried stone vaults reserved for chieftains, their family and their primary retainers. A third burial mound classification, the *langi*, consists of a single or multiple platforms, supported by stone retaining walls (McKern 1929). They were reserved for the Tu'i Tonga (McKern 1929). To-At-1 and To-At-2, were chosen for excavation due to their representativeness of the

two burial mound classifications observed during the survey (Davidson 1969). In addition to investigating the structure of these mounds, the main aim of this excavation was to test the hypothesis that the presence of white sand is indicative of burials (Davidson 1969).

To-At-1

To-At-1 was the first mound excavated. Being a relatively small mound of only 40 metres in diameter and 80cm in height, To-At-1 was chosen as a representative of the *tanuanga* type burial mound (Davidson 1969). Three trenches were opened up across the mound, with a total of 27 square metres excavated (Figure 3.3) (Davidson 1969).

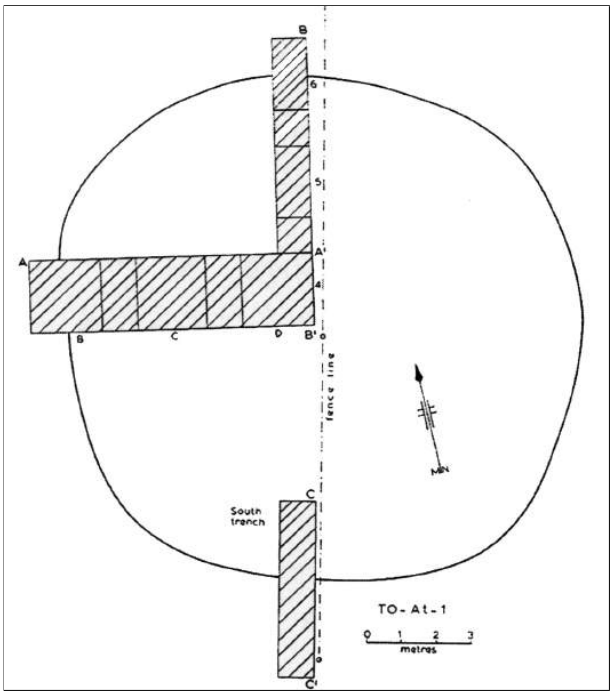

Figure 3.3. Map showing excavation plan of To-At-1 (Buckley 2016:47).

Four periods of occupation were evident in the stratigraphy (Davidson 1969). The earliest occupation of the site is evidenced by a series of postholes most likely used for yam planting (Davidson 1969). A short lived domestic occupation appears to have occurred next as evidenced by a series of postholes and two firepits (Davidson 1969). It was after the end of this domestic occupation that To-At-1 was first used for burials

(Davidson 1969). Postholes suggest a house or other special structures were built over the burials during this time (Davidson 1969). In its final period of use, another shallower mound was constructed over the centre of the mound for additional burials (Davidson 1969).

A total of 38 interments, containing 42 individuals were excavated from To-At-1 (Buckley 2016; Davidson 1969). The majority of these individuals were completely excavated, however as the excavation strategy was designed to investigate the structure of the mound rather than for the excavation of skeletal remains, some burials extended into the baulks and were only partially excavated (Buckley 2016; Davidson 1969). There was also a high number of burials in a relatively small amount of space meaning there was comingling of skeletal remains (Davidson 1969). Despite this, overall preservation is good.

To-At-2

Visibly larger and with a surrounding ditch, To-At-2 was chosen for excavation as a representative of the *faitoka* burial mound style (Davidson 1969). A one metre wide trench was excavated through the centre of the mound, with extensions excavated at either end (Figure 3.4) (Davidson 1969). Two additional trenches were excavated at the east and west boundaries of the mound, and two test pits were excavated in areas surrounding the mound where surface potsherds were numerous (Davidson 1969).

Evidence of a posthole, shells and fragments of turtle bone suggests the earliest occupation of To-At-2 was domestic (Davidson 1969). At this time To-At-2 was a shallow pit (Davidson 1969). At the end of this domestic use, To-At-2 was infilled and burial pits were dug into the new surface (Davidson 1969). A mound was then constructed using spoil from a ditch that was dug around the mound (Davidson 1969). Burial pits were dug into this mound (Davidson 1969). A second ditch was then dug and the spoil used to enlarge the mound for further burials (Davidson 1969). In its final period of use, Additional burials were dug into the slopes of the mound and into the fill of the outer ditch (Davidson 1969).

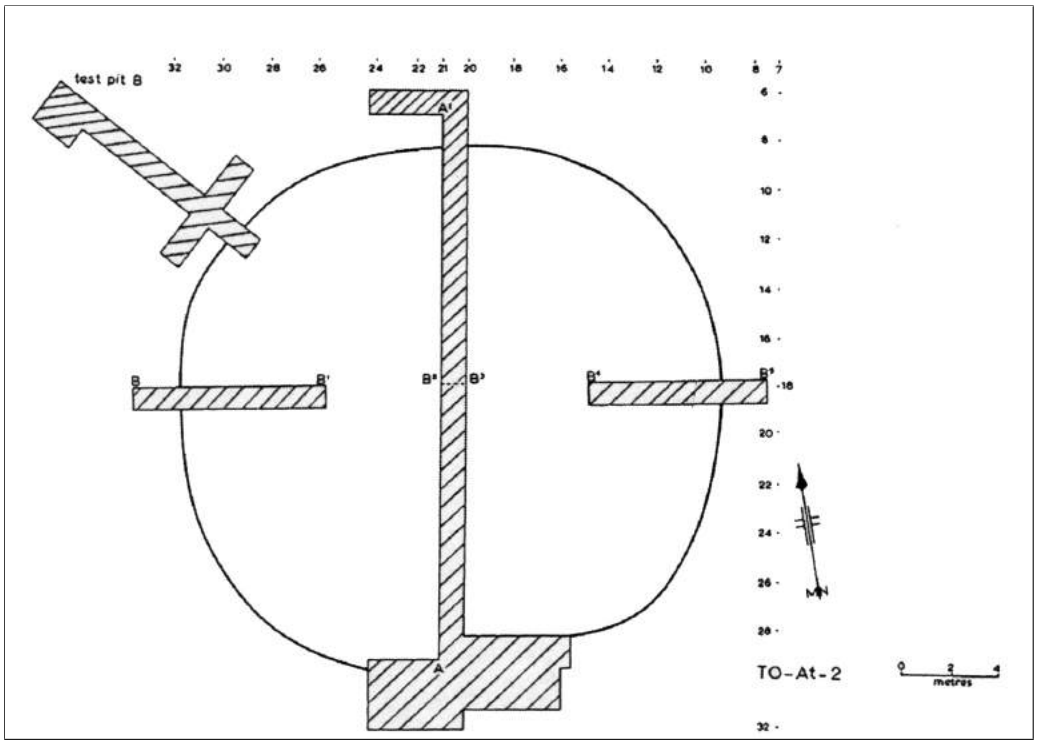

Figure 3.4. Map showing excavation plan of To-At-2 (Buckley 2016:47).

A total of 41 interments, containing 52 individuals were excavated from To-At-2 (Buckley 2016; Davidson 1969; Stantis 2015). Again, the excavation strategy was poorly designed for the recovery of skeletal remains (Davidson 1969). Preservation also appears to vary more greatly in the bones excavated from To-At-2 than To-At-1. This is particularly apparent in a number of badly crushed remains excavated from the outer ditch (Davidson 1969). Erosion also caused problems for the excavation of To-At-2, as it caused the sides of the trenches to crumble towards the end of the excavation (Davidson 1969). This erosion revealed previously unseen burials (Davidson 1969). Loose bone from these burials was collected but not assigned to any particular burial (Davidson 1969). As a result there are a number of "unnumbered" individuals (n=26) within the 'Atele skeletal sample that lack context (Stantis 2015). As erosion does not appear to have had a similar effect on To-At-1, it is most likely that all "unnumbered" individuals come from To-At-2.

3.1.4 Previous bioarchaeological research

The first bioarchaeological research performed on the ‘Atele sample was by Pietrusewsky (1969), who carried out a detailed osteological analysis of all the individuals excavated from the ‘Atele mounds, including the “unnumbered” individuals. During this analysis, Pietrusewsky (1969) made estimations for age, sex and stature. Assessments were also made of metric and non-metric skeletal and dental traits, and any evidence of palaeopathology (Pietrusewsky 1969).

A detailed examination of health and disease within the ‘Atele sample was performed by Buckley (2000; 2001; 2006; 2016). The main aim of this research was to assess the role of infectious disease on the health of prehistoric Pacific island populations (Buckley 2016). This was achieved by comparing the ‘Atele sample, which is outside of the Pacific malarial zone, to a sample from Taumako in the Solomon islands, which is within the Pacific malarial zone (Buckley 2016). Demographic profiles were compiled for each sample to test whether the presence of malaria in Taumako affected mortality rate (Buckley 2016). Non-specific dental and skeletal markers of childhood stress were recorded to investigate the impact malaria had on growth (Buckley 2016). Skeletal markers of iron-deficiency anaemia and infectious disease were also recorded to investigate interpopulation difference in regards to disease load (Buckley 2016). Very few differences were seen in the demographic profiles of each population however the individuals from Taumako displayed significantly more markers of childhood stress (Buckley 2016). The skeletal markers of iron-deficiency anaemia were also more numerous and more severe within the Taumako population than they were for ‘Atele (Buckley 2016). In regards to infectious disease, a similar proportion of proliferative skeletal lesions was seen between the two samples, but like the results for iron-deficiency anaemia, the severity of the lesions was greater for Taumako (Buckley 2016). For ‘Atele, a more non-specific pattern of skeletal lesions was observed (Buckley 2016). Buckley (2000) performed a differential diagnoses on the skeletal lesions observed on the skeletal remains of 17 subadults from the ‘Atele population. Possible diagnoses included iron-deficiency anaemia, haematogenous osteomyelitis, congenital syphilis, yaws, scurvy, hypervitamintosis A, trauma and

Caffey's disease (Buckley 2000:481). Most likely a combination of these aetiologies were responsible for the skeletal lesions observed (Buckley 2000).

Two additional non-specific indicators of stress, cortical index and maximum femoral length, were examined by Robb et al. (2012). Measurements of cortical index and maximum femoral length were taken from the 'Atele sample and then compared to the Taumako sample and a sample from Teouma in Vanuatu (Robb et al. 2012). The aim was to investigate adaptive responses across Pacific islands that differed both temporally and environmentally (Robb et al. 2012). The results of this study showed no significant differences between the samples, arguing against differential adaptive responses (Robb et al. 2012).

Entheseal changes and skeletal evidence for osteoarthritis within the 'Atele sample was examined by Foster (2012) as part of a larger study assessing whether there were gendered divisions of labour in prehistoric Southeast Asian and Pacific populations. While gendered differences in entheseal change and osteoarthritis prevalence was seen clearly within the Southeast Asian samples, very few gendered differences were seen in the Pacific samples, including 'Atele (Foster 2012). This is interesting as it goes against ethnographic and historical information for Tonga, which describe gendered differences in the roles of men and women (Malm 2007; Mariner and Martin 1981; Morton 1996)

Evidence of trauma within several Pacific island samples was investigated by Scott (2008), with the results for the 'Atele sample being published in Scott and Buckley (2014). The aim of this research was to investigate whether skeletal trauma within the 'Atele sample was reflective of deliberate violence due to the volatile nature of the Chiefdom period (Scott and Buckley 2014). Interestingly, the pattern of trauma observed more closely resembled that of ritualised trauma or sporting activities, both of which have been reported in ethnographic and historical literature (Bott 1982; Ferdon 1987; Mariner and Martin 1981; Mills 2009; Suren 2009).

The use of stable isotope analysis to investigate the diet of the 'Atele individuals was first applied by (Quinn 1990). The results of this study suggested that the 'Atele individuals had a mixed diet of marine/reef resources and terrestrial plants (Quinn

1990). This was consistent with earlier research by Evans (1987), who analysed oral health indicators (wear, periodontitis and caries) and saw a pattern in these indicators that suggested the ‘Atele individuals were horticulturalists, consuming a diet low in fibre, low in grit, and which contained some cariogenic foods.

In a more recent study, Stantis (2015) combined stable isotope analysis with the assessment of oral indicators of diet to achieve a fuller understanding of the ‘Atele diet. Stantis (2015) observed a significant difference in diet between the sexes, with females displaying significantly lower δ^{15} nitrogen values than males (Stantis 2015). A similar result was seen in the subadults of the sample, with subadults displaying δ^{15} nitrogen values slightly lower than the adults (Stantis 2015). It was argued that this could reflect cultural restrictions in regards to access to animal protein, a lack of which could have had serious repercussions for health (Stantis 2015). This hypothesis is supported by the higher δ^{15} nitrogen values observed in the childhood diet of the adults who survived childhood stress when compared to the childhood diets of the subadults who did not (Stantis 2015). Another interesting result was the significantly higher δ^{15} nitrogen values observed for To-At-2 compared to To-At-1, suggesting the To-At-2 individuals were consuming more animal protein (Stantis 2015). This is interesting as no prior study had found evidence of difference between the mounds. Despite the sex based differences observed in the stable isotope analysis, no significant differences where observed in the oral indicators (Stantis 2015). This overall lack of sex-based differences conflicts with the trend seen in the prevalence of dental caries within societies practicing agriculture where women display a higher prevalence of dental caries than men (Lukacs and Largaespada 2006; Willis and Oxenham 2013). The discrepancy between the oral indicators of diet and the stable isotope analysis was revisited by Stantis et al. (2015) who suggested that the differences in diet may have been too sensitive to impact oral health.

One of non-specific dental markers of childhood stress examined by Buckley (2016) was linear enamel hypoplasia (LEH). LEH is a hypoplastic developmental defect of enamel (DDE) that forms when matrix secretion is disturbed during enamel formation (Hillson 1996). Disturbances to matrix mineralisation can also result in

hypomineralised defects, characterised by discoloured areas of reduced mineral density (Hillson 1996). Hypomineralised defects are often excluded from investigations into growth disturbance as many taphonomic processes can leave behind similar discolouration (Alexandersen et al. 1998). Whether or not this is an issue for the discolourations observed within the ‘Atele sample was explored by Farah et al. (2016). In this study, x-ray microtomography was used to measure the mineral density of affected teeth (Farah et al. 2016). Reduced mineral density was observed on the teeth displaying discolouration’s, indicating that this discolouration is in fact developmental rather than the result of a taphonomic process (Farah et al. 2016). A comprehensive assessment of all DDE present in the ‘Atele sample was performed by Barker (2016). This investigation found a significant difference in DDE prevalence between the two mounds, with a significantly lower prevalence rate and variety of DDE observed within the To-At-1 (Barker 2016). Another interesting result was women having a significantly higher prevalence rate than men in both mounds (Barker 2016). These results are consistent with what would be expected of these groups if, as suggested by Stantis (2015), the decreased consumption of animal protein was having a negative impact on health.

3.2 Anatomy museum collection

As mentioned above, a foetal femur (O2.D44) from the W.D. Trotter Anatomy Museum was also selected for this assessment as a non-burial depositional context control. The W.D. Trotter Anatomy Museum, founded between 1875 and 1881, is part of the University of Otago Medical School (Page 2008). Very little information exists regarding the provenance of the skeletal material of approximately 27 individuals that makes up the foetal and infant component of this collection (Southorn 2019). It is most likely that they were acquired during the early years of the Museum (Southorn 2019).

3.3 Conclusion

This chapter has provided discussion of the geographical, climatic, and cultural history context of the ‘Atele skeletal collection, as well as a discussion of its excavation and the research that has been performed on it since its excavation. This research covers a number of aspects of the lifestyle of the ‘Atele population including health, trauma, activity, and diet. This research provides a valuable foundation from which to use the results of this assessment to further investigate the health of the ‘Atele population.

4 Methods

An inventory of the skeletal and dental material of all the ‘Atele infants showed that eight of eleven infants aged from around birth to one year of age had bones appropriate for micro-CT imaging. A foetal femur (O2.D44) from the W.D. Trotter Anatomy Museum was also selected for this assessment. This chapter provides a discussion of the methods used to calculate age-at-death estimations for these nine infants. This is followed by a discussion on Micro-CT imaging, outlining why it was selected for this assessment and the advantages of this method over other histological techniques. This chapter concludes with a discussion on the Oxford Histological Index (OHI), the most commonly used method to assess bone for microbial bioerosion.

4.1 Age-at-death estimations

It is important to define the age terminology used in this thesis. The terminology defined by Lewis (2007:2) is used in this thesis (Table 4.1). A premature birth is defined as a perinatal birth earlier than 37 gestational weeks (Halcrow et al. 2008:384). These terms are intended to provide researchers with a consistent, physiological frame of reference when discussing age-at-death estimations. It must be acknowledged that there are many socio-cultural factors that determine how a population will actually define an infant, child, or adult, and this can differ from the definitions provided below (Halcrow and Tayles 2011; Lewis 2007; Lewis 2019).

Table 4.1. Age categories used in this thesis and their definitions. Adapted from Lewis (2007:2).

Age terminology	Definition
Embryo	Zero to eight gestational weeks
Fetus	Eight gestational weeks to birth
Stillborn	Foetal death after 28 gestational weeks
Perinate	Infant born alive from 24 gestational weeks to seven postnatal days
Premature	Perinatal birth under 37 gestational weeks
Neonate	Birth to 27 postnatal days
Post-neonate	28 postnatal days to one year of age
Infant	Birth to one year of age
Child	One to 14.6 years of age
Adolescent	14.6 to 17 years of age
Subadult	$\leq$ 17 years of age
Adult	> 17 years of age

Age estimations for the ‘Atele skeletal series were previously calculated by Pietrusewsky (1969) and Buckley (2016). For objective two, independent age estimations for the infant individuals of To-At-1 and To-At-2 were calculated. Age estimation is a crucial part of any bioarchaeological investigation. Correlates are often seen between age-at-death and the living standards, economic status, adaptability, health, and cultural practices of past populations for which there is no written record (Halcrow and Tayles 2011; Halcrow et al. 2007; Lampl and Johnston 1996; Lewis 2007; Lewis 2019; Tocheri et al. 2005). Subadult age estimation can also provide important information about growth rates, weaning practices, maternal health and infanticide (Lampl and Johnston 1996; Lewis 2007). Accurate infant age-at-death estimations are particularly important given the vulnerability of these individuals who are completely dependent on their wider community to supply them with the means to survive (Lewis 2007; Lewis 2019). It is important then, for age-at-death estimations to be as accurate as possible.

Calculating age-at-death estimations for archaeological skeletal remains involves observing morphological features within the skeleton and using these features to assign a physiological age-at-death (Saunders 1992; Ubelaker 1989). Physiological age is an individual’s biological age and it refers to how developed an individual is at the time of death (Scheuer and Black 2000). An individual’s known age, also known as their chronological age, refers to their calendar age (Scheuer and Black 2000). Due to the nature of archaeological skeletal samples, it is not often possible to establish known age-at-death from skeletal remains alone.

Aging standards developed using historical populations of known age-at-death, or even living populations, are used to estimate physiological age-at-death for archaeological populations (Lampl and Johnston 1996). A major issue that arises with the use of standards developed using populations of known age-at-death is that physiological age-at-death often varies from known age-at-death (Lampl and Johnston 1996). Although researchers usually acknowledge this, there is no way to know how much it varies (Lampl and Johnston 1996). Another issue is that these standards are specific to the populations used to develop them, and these populations often come from very different environmental, socio-economic, and chronological contexts to the

archaeological populations they are applied to (Lampl and Johnston 1996). At present, the majority of aging standards have been developed using modern North American and European populations (Lampl and Johnston 1996; Saunders 1992). Few standards exist for non-Caucasian populations, and there are no standards available at present for the Pacific. This is particularly a concern for this study as advanced dental formation and eruption has been observed in Pacific Island children (Fry 1976; Te Moananui et al. 2008). These standards also assume that the growth patterns of modern populations do no differ significantly from the growth patterns of past populations, something that cannot be tested when known age cannot be confirmed (Bocquet-Appel and Masset 1982; Hoppa 2000).

The methods used to age adult and subadult remains vary. Most commonly, adult age-at-death is calculated by observing morphological changes to the pubic symphysis and auricular surface of the pelvis, as well as through observing fusion of the cranial sutures and epiphyses (Buikstra and Ubelaker 1994). Evidence of degenerative changes, such as dental wear and osteoarthritis, can also be used in the estimation of adult age-at-death (Buikstra and Ubelaker 1994). Epiphyseal fusion, dental formation, dental eruption and diaphyseal length can be used to estimate age-at-death older subadult remains (Buikstra and Ubelaker 1994; Ubelaker 1989). In particular, observation of the formation and eruption of the dentition, and diaphyseal length are the principal methods used to the estimate age-at-death of infants. These methods are discussed below.

4.1.1 Dental formation and eruption

Dental formation

Dental formation begins with initial tooth germ development around 10 weeks after fertilization and covers the long prenatal and postnatal period of crown and root formation (Hillson 1996). Dental formation ageing standards are concerned with the morphological changes that occur during crown and root formation, which begins with the mineralisation of the deciduous central incisors around 14 weeks gestation and ends

with root completion of the permanent third molars in the late teens, early twenties (Hillson 1996).

Two of the most commonly used standards for dental formation are Moorrees et al. (1963a; 1963b). Moorrees et al. (1963a) used radiographs of 246 subadult individuals of known sex, taken as part of Fels Longitudinal Study, to define several developmental stages for deciduous mandibular canines and molars. Moorrees et al. (1963b) supplemented these radiographs with incisor radiographs from an additional 99 individuals from another longitudinal study to define formation stages for permanent maxillary and mandibular incisors, and mandibular canines, premolars, and molars. The mean age at which each stage of formation occurs, as well as standard deviations, were calculated for each tooth studied.

More recently, research has gone into developing regression formulas based on the relationship between tooth length and age. Regression formulas to calculate age-at-death from all the deciduous dentition and the permanent incisors, canines, and first molars were developed by Liversidge et al. (1993) based on measurements of tooth length from 63 infants and young children from the crypt of Christ Church (Spitalfields, London) skeletal collection. Coffin plates and parish records provided known age and sex for these individuals (Liversidge et al. 1993). The ageing methods discussed above (and those discussed below regarding dental eruption), where age-at-death is estimated by assigning a morphology-based stage, can be very subjective, determined by the training, experience, and knowledge of those applying the methods (Liversidge et al. 1993). The continuous nature of dental formation also means that teeth do not always fit nicely into the stages defined in standards and are instead, placed in the "next best" option, over- or under-estimating age (Liversidge et al. 1993). This subjectivity is only increased when teeth are found in isolation and there is nothing to compare it to (Liversidge et al. 1993). Standards such as this, that are based on direct measurements can provide a more objective alternative, thereby increasing the accuracy of estimations (Liversidge et al. 1993).

The accuracy of this method for developing deciduous teeth was tested by Cardoso (2007) using the dentition of 30 subadult individuals of known sex and age-at-death from the Lisbon Collection (Cardoso 2007). Age-at-death was estimated for these

individuals using the formula developed by Liversidge et al. (1993), and this estimate was compared to the known chronological age for each individual (Cardoso 2007). Cardoso (2007:18) found a high accuracy between the estimates provided using the Liversidge et al. (1993) formulae and the known chronological ages for the Portuguese subadults, with average differences in age of 0.20 and -0.14 years when just a single tooth was used, and 0.06 years when all available teeth were used. It was evident that the formula tended to overestimate the age of deciduous molars, while underestimating the age of deciduous anterior teeth (Cardoso 2007). This result was only statistically significant for the second molar, so these overestimations and underestimations may cancel one another out (Cardoso 2007).

Cardoso (2007) also highlighted an issue with the Liversidge et al. (1993) standards, in that apart from the permanent lateral incisor, the data for upper and lower teeth is combined. Combining the data for the upper and lower teeth could bias age estimations because these teeth are known to develop and erupt at different rates (Hillson 1996). Liversidge et al. (1993) did not specify why the data for upper and lower teeth was combined but did mention that the data for the permanent lateral incisor was kept separate due to an apparent difference in the timing of initial mineralisation. Cardoso (2007) believes that the bias caused by combining the data for the upper and lower teeth will only cause significant variation from chronological age if the formulae is applied to only the upper or lower teeth.

Dental eruption

Dental eruption refers to the emergence of teeth from their bony crypts, through the alveolar bone and gums to occlude with opposing teeth (Hillson 1996). This occurs within the first two years of life for the deciduous dentition, and between five to eight years to age for the permanent dentition (Hillson 1996). Eruption is complete with the eruption of the third molars in the late teens to early twenties (Hillson 1996).

Schour and Massler (1941) developed a popular chart documenting dental eruption from five month *in utero* to adulthood (cited in: Hillson 1996). Little is known about the sample on which this chart was developed, with suggestions that it is based on earlier research by Logan and Kronfeld (1933), Kronfeld (1935), and Kronfeld and

Schour (1939) (AlQahtani et al. 2014; Hillson 1996). A revised version of the Schour and Massler (1941) chart was developed by Ubelaker (1989), combining the results of many investigations into the timing of dental formation and eruption in American Indian and other "non-white" populations.

An issue with dental eruption standards such as these is that the definition of eruption is often used incorrectly in clinical research to refer only to the appearance of the tooth crowns through the gum (Demirjian 1978). In fact, as mentioned above, dental eruption covers the entire process by which teeth emerge from their bony crypts and move through the alveolar bone and gums to occlude with opposing teeth (Hillson 1996). The nature of archaeological skeletal remains makes it difficult to determine whether teeth have erupted through the gum, with it often only possible to determine whether teeth have erupted through the alveolar bone (Halcrow et al. 2007). This is a concern for archaeological samples because alveolar emergence and gingival emergence are believed to happen at different times (Hulland et al. 2000; Konigsberg and Holman 1997).

4.1.2 Diaphyseal length

While the highly mineralised nature of dentition means that it preserves well in the archaeological record, developing tooth buds are small, fragile, and can be missed in archaeological excavations (Halcrow et al. 2008; Tocheri et al. 2005). When dental formation and eruption cannot be used for age-at-death estimations, measurements of the length of growing long bones (diaphysis) can be used instead (Saunders 1992; Ubelaker 1989). Bone growth during the perinatal period is rapid, leading to large differences between age categories (Halcrow et al. 2008; Mays 2010). Although skeletal growth is considered more variable than dental development, with greater impact from environmental factors, growth is so rapid during the perinatal period that these factors are thought to only greatly impact age-at-death estimations of older children (Halcrow et al. 2008; Mays 2010). Factors such as maternal health also have to be very severe to cause growth retardation in foetuses (Mays 2010).

Fazekas and Kósa (1978) provided one of the most commonly applied standards for estimating fetal age-at-death from three lunar months to term. This standard was developed using 138 Hungarian foetuses of forensic origins whose age-at-death is unknown (Fazekas and Kósa 1978). These foetuses were sorted into age groups from three lunar months to term (at 0.5 month intervals) based on crown-heel length (Fazekas and Kósa 1978). These crown-heel lengths were then correlated with long bone lengths for these individuals (Fazekas and Kósa 1978). While a relationship between body length and age is recognised, standards such as this, developed using individuals whose known age-at-death is unknown, often become very circular in nature, producing inaccurate age-at-death estimations (Cunningham et al. 2016; Lewis 2007; Lewis 2019).

Merchant and Ubelaker (1977) developed tables where maximum diaphyseal length of the humerus, radius, ulna, femur, tibia, fibula and maximum iliac breadth were correlated with age using measurements from 193 protohistoric Arikara individuals, excavated from the Mobridge Site cemetery in South Dakota (Merchant and Ubelaker 1977). Age-at-death estimations for these individuals were calculated using the Moorrees et al. (1963a; 1963b) standards mentioned above, with the 193 individuals studied being placed into yearly age categories from newborn to 18 years of age (Merchant and Ubelaker 1977). The mean, standard deviation, and ranges of maximum long bone length and maximum iliac breadth was then calculated for each age category. This is one of the few standards developed on a sample not of known age-at-death, making it more relevant to archaeological samples than some of the other standards developed using samples of known age-at-death. A disadvantage of this standard is that there are large discrepancies in the number of individuals contributing to each age group (Lewis 2007). It is recommended that this standard only be used to calculate age-at-death estimations for individuals aged from birth to 2.5 years of age, as the greatest number of individuals contributed to this age group (Lewis 2007).

Measurements of long bone length from known age-at-death subadults have also been used to develop regression equations to calculate age-at-death from lone bone length (Carneiro et al. 2013; Scheuer et al. 1980). Scheuer et al. (1980) used maximum femora, tibiae, radii, ulnae, and humeral diaphyseal lengths measured from X-ray and radiograph images of 82 Portuguese fetuses and neonate infants of known age-at-death

in the development of their regression equations. These images were sourced from The Bristol Royal Hospital for Sick Children and The London University Institute of Child Health (Carneiro et al. 2013). Carneiro et al. (2013) used maximum femora, tibiae and humeral diaphyseal length measured from the autopsy radiographs of 100 Portuguese fetuses of known ages-at-death between 13 and 40 gestational weeks to produce a single regression equation for each bone. Carneiro et al. (2013) also provided reference tables of the mean, standard deviation and the 95% confidence intervals at 5-week intervals from 13 to 37+ weeks gestation for each of the bones studied.

4.1.3 Ageing methods used in this analysis

Age-at-death estimations were calculated for the nine individuals included in this analysis using a combination of dental formation, dental eruption, and diaphyseal length standards. The dental formation and eruption standards used were Liversidge et al. (1993), Moorrees et al. (1963a), Moorrees et al. (1963b), and Ubelaker (1989). The diaphyseal length standards used were Carneiro et al. (2013), Merchant and Ubelaker (1977), and Scheuer et al. (1980). While dental formation and eruption is more strongly controlled by genetics, preference was given to diaphyseal length due to the difficulties surrounding the recovery of small developing tooth buds (Lewis 2007; Lewis 2019). Using a combination of these methods allows comparisons to be made between dental age and skeletal age (Lewis 2019). Discrepancies in these ages can help identify small for gestational age infants, important indicators of maternal health (Lewis 2007; Owsley and Jantz 1985).

4.2 Micro-CT imaging

To assess the infants of the ‘Atele skeletal collection for microbial bioerosion the internal microstructure of bone needed to be visualised. Traditionally, imaging of the internal microstructure of bone is achieved through histological techniques that require the transverse sectioning of bone for microscopic examination (Booth 2017; Booth 2020; Gartner 2017; Miszkiewicz and Mahoney 2017; Rühli et al. 2007). A number of special preparations, including fixation, dehydration, embedding, and

polishing, are also required before these sections can be mounted onto microscope slides (Booth 2017; De Boer et al. 2013; Gartner 2017; Miszkiewicz and Mahoney 2017; Schultz 2001). The end result is a thin section ranging from 50 to 100µm in thickness that can be magnified as much as 60x to 100x using a high-powered microscope (Miszkiewicz and Mahoney 2017). While sampling techniques can be minimally invasive and thin sections can be stored for use in later analyses (Miszkiewicz and Mahoney 2017), the required excision and special preparations of sections makes histological techniques inherently destructive.

Micro-computed tomography, also known as micro-CT (µCT), represents a non-destructive method for the imaging of the internal microstructure of bone (Booth 2020; Booth et al. 2016; Dal Sasso et al. 2014; Rühli et al. 2007). Similar to the computed axial tomography (CAT) and computed tomography (CT) commonly used in hospitals but on a much smaller scale, micro-CT scanning involves an x-ray source and detector collecting sectioned images of an object which can then used to reconstruct a three-dimensional (3D) model of the object (Bruker 2019; Smith et al. 2013).

4.2.1 Advantages of micro-CT imaging archaeological human remains

Archaeological skeletal remains are often fragmentary and damaged, so it is critical to use investigative techniques that limit further destruction (Rühli et al. 2007). One advantage of micro-CT for the imaging of the internal microstructure of bone is that no special preparations are required before a sample can be scanned, so micro-CT can be considered non-destructive (Booth 2020; Booth et al. 2016; Rühli et al. 2007).

Resolutions as high as 2-3µm can be achieved using micro-CT (Smith et al. 2013). A disadvantage of this is that small samples are required to achieve such high resolutions. The specifications of the scanner used will dictate the size of the objects that can be scanned. The small size of foetal and infant skeletal remains means they are well suited to micro-CT imaging (Booth et al. 2016). Adult bones can still be scanned but may require sections to be excised, or for fragments to be preferentially targeted (Booth et al. 2016). As no special preparations are required before a sample can be micro-CT scanned, even if sectioning is required it is considered minimally invasive

(Booth et al. 2016). The specifications of the scanner used in this project are outlined below.

Another advantage of using micro-CT imaging is the large volume of information that is captured, with the ability to capture thousands of images during a single scan. Traditional histological techniques produce only a single cross-section of a sample taken from a targeted location (Booth et al. 2016). Micro-CT imaging captures images along the entire length of a sample, allowing the microstructure of the entire sample to be visualized and assessed (Booth 2020; Booth et al. 2016; Dal Sasso et al. 2014; Rühli et al. 2007). Single cross-sections of bone do not necessarily provide a representative sample of the microstructure of the entire bone it came from. Research has shown great variability in the level of diagenetic change that can occur to burials from the same site, from different bones of the same skeleton, and in different locations within a single bone (Booth et al. 2016; Hanson and Buikstra 1987; Hedges 2002).

4.2.2 Sampling

Previous analyses of microbial bioerosion in human skeletal remains (Booth 2016; Jans et al. 2004; Nielsen-Marsh and Hedges 2000; White and Booth 2014), have preferentially targeted femora for analysis due to the high cortical bone content, robusticity, and preservation potential of femora compared to other bones. This was tested by Dal Sasso et al. (2014), who compared femora, rib, and cranial fragments to see which was more suitable to the micro-CT analysis of microbial bioerosion. The femur was found to be the most suitable, as the higher percentage of trabecular bone in the rib and cranial fragments made them more porous and more prone to infilling with secondary calcite and quartz sand grains from the burial environment, contributing to poorer preservation (Dal Sasso et al. 2014:36). When femora are not available other bones can be used, other long bones being preferable (Jans et al. 2004).

4.2.3 Micro-CT methods employed in this analysis

Ideally, femora would have been targeted in this project for consistency with previous research, however no complete or fragmentary femora of an appropriate size for micro-CT imaging were available for the ‘Atele infants included in this study. Instead, other long bones were targeted. Where there were no appropriate long bones available, ribs were targeted due to the close proximity of the rib cage to the gut and the observed relationship between endogenous gut bacteria and the formation of non-Wedl MFD (Chapter 2.3.2). The bones targeted for each infant included in this analysis are summarised in Table 4.2 below.

A SkyScan 1172 scanner (Bruker-MicroCT, Kontich, Belgium), housed in the Otago Micro and Nanoscale Imaging unit (OMNI) at the University of Otago, was used in this analysis according to the following parameters: source voltage of 50kV, source current of 200µm, 8.7µm image pixel size, an aluminum 0.5mm filter, rotation step of 0.4°, 180° rotation, frame averaging of 4, flat field correction, geometrical correction and median filtering. The x-rays were detected by a Hamamatsu 10Mp camera, with a 12-bit CDD. The x-ray images were reconstructed using Nrecon software (version 1.6.10.2, SkyScan, 2011), using a modified Feldkamp cone-beam algorithm. The reconstructions were according to the following parameters: file type TIF, image height of 840 pixels, image width of 840 pixels, average pixel size of 8.7µm, ring artefact correction of 10%, and beam hardening correction of 50%. This scanner can scan objects measuring up to 55x55x55mm (OMNI n.d).

Table 4.2. Summary of which bone was scanned for each individual in the ‘Atele infant collection and the foetal femur (02.D44) from the Anatomy Museum.

Individual	Bone scanned
02.D44	Right femur
To-At-1/1b	Left radius fragment
To-At-1/5	Misc. rib fragment, side unknown
To-At-1/13b	Misc. rib fragment, side unknown
To-At-1/16	Radius shaft fragment, side unknown
To-At-1/29c	Radius fragment, side unknown
To-At-2/2	Distal ulna fragment
To-At-2/14	Misc. rib fragment
To-At-2/17	Misc. rib fragment

4.3 Microbial bioerosion assessment

The most commonly used method to assess the level of bioerosion within modern and archaeological bone samples is the Oxford Histological Index (OHI). Developed by Hedges et al. (1995), the OHI describes the percentage of unaltered bone microstructure in a bone section using a zero to five scoring system, where zero means no original features are identifiable, and five means the microstructure is virtually indistinguishable from that of fresh bone. While the OHI is subjective, Hedges et al. (1995) found interobserver error to be no more than one unit when blind tests where run. Prior to the development of the OHI there was no method for the quantification of bioerosion, and most analyses focused on identifying the presence and type of bioerosion within bone sections (Bell 1990; Bell et al. 1991; Bell et al. 1996; Hackett 1981; Hanson and Buikstra 1987; Hedges et al. 1995; Yoshino et al. 1991). Scoring systems have been used to indicate the type of histological degradation seen in bone sections. Garland (1987) for example, studied the histological preservation of sections from 76 bones and bone fragments from various burial environments dating from *ca.* 9000 BC to the early 20th century. Each section was graded into four categories based on their main histological appearance: 1) 'normal" histological appearance, 2) destructive changes, 3) inclusions, and 4) infiltrations (Garland 1987). The 'destructive changes' category is further divided into a) generalised destruction, and b) focal destruction. Bioerosion fell into the 'focal destruction' subcategory (Garland 1987). The OHI has since been modified by Millard (2001) to allow for more mid-range levels of histological preservation (Booth 2014).

The applicability of the OHI to micro-CT images of bone was tested by Booth et al. (2016). As discussed in the previous chapter, Booth et al. (2016) assessed ten Romano-British perinates and three adults for bioerosion using micro-CT. Mid-shaft femoral fragments from the three adult individuals had already been excised and assessed for bioerosion using transmitted light microscopy by Jans et al. (2004). Micro-CT images of these samples were created and reassessed for bioerosion (Booth et al. 2016). Comparable OHI values were seen between the transmitted light microscopy assessment and the Micro-CT assessment, suggesting that the OHI is an appropriate method to quantify bioerosion when samples are imaged using micro-CT (Booth et al.

2016). When assessing the infants for bioerosion Booth et al. (2016:127) noticed that the contrast between bioeroded and unbioeroded bone was not as distinct as in the micro-CT images captured of the adult samples. This gave the bioeroded bone in the infant micro-CT images a “mottled” appearance (T. Booth, pers. comm.). This may be due to the lower mineralisation of infant woven bone compared to adult lamellar bone (Booth et al. 2016). This did not impact the detection of bacterial bioerosion within these samples, which was found to be located mainly in the sub-endosteal and sub-periosteal regions of each bone sample affected, with no evidence of bioerosion being observed in the periosteal and endosteal margins (Figure 4.1) (Booth et al. 2016). The lack of non-Wedl MFD within the periosteal and endosteal margins is a commonly observed pattern for non-Wedl MFD (Bell et al. 1996; Booth 2014; Booth et al. 2016; Hanson and Buikstra 1987; Hollund et al. 2012; Jans et al. 2004; White and Booth 2014).

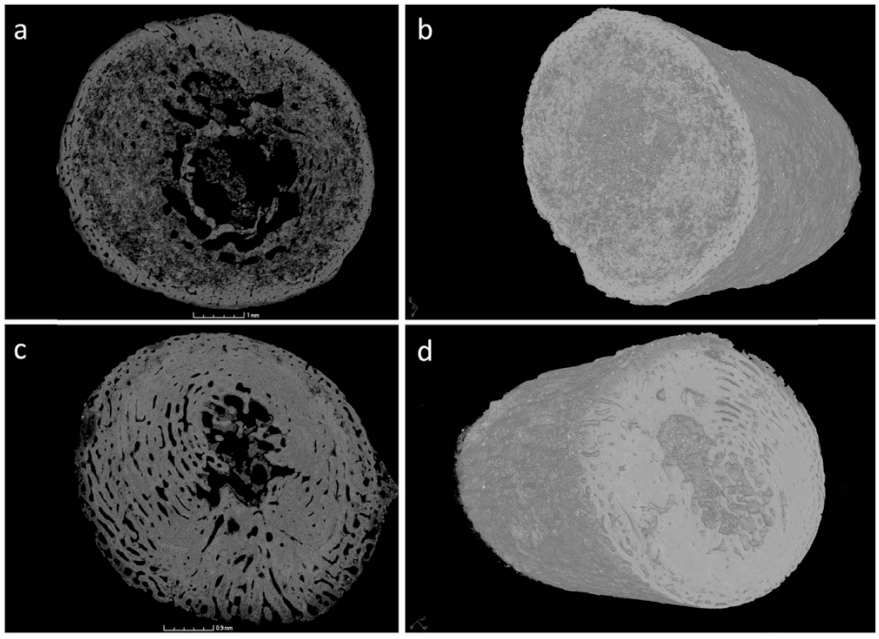

Figure 4.1. Example of micro-CT images captured by Booth et al. (2016:131). A) and B), infant IS2 with extensive evidence of non-Wedl MFD (OHI = 1). C) and D), infant IS10 with no evidence of non-Wedl MFD (OHI = 5).

4.3.1 Methods used in this analysis

In this project, the modified version of the OHI developed by Millard (2001:640) was used (Table 4.3). As mentioned earlier, one benefit of using micro-CT imaging is the volume of sections that is produced for each sample, allowing the whole sample to be visualised and assessed rather than just a single section (Booth 2020; Booth et al. 2016; Dal Sasso et al. 2014; Rühli et al. 2007). To test for variation in OHI score within a single bone, multiple images were assessed. For example, of the 1047 images captured for infant To-At-1/1b, the first image in the series was assessed, followed by the 100th, 200th, 300th… … 800th, 900th, and 1000th images in the series. The last image in the series was also assessed, bringing the total number of images assessed to twelve. A final score for the whole bone was calculated as the average of these scores. Non-Wedl MFD was identified in accordance to Booth et al. (2016): when the contrast between areas of low and high density caused the woven bone within the samples to become "mottled" in appearance, and when the periosteal surface was well preserved compared to the rest of the bone.

Table 4.3. Oxford Histological Index. Adapted from Millard (2001:640).

Index	Approx. % of intact bone	Description
0	< 5	No original features identifiable, other than Haversian canals
1	<15	Small areas of well-preserved bone present, or some lamellar structure preserved by pattern of destructive foci
2	< 50	Some lamellar structure preserved between destructive foci
3	>50	Some preservation of some osteocyte lacunae
4	>85	Only minor amounts of destructive foci, otherwise generally well preserved
5	>95	Very well preserved, virtually indistinguishable from fresh bone

5 Results

The following chapter outlines the results of the age-at-death estimations, micro-CT scanning, and microbial bioerosion assessments performed on eight infants from the ‘Atele skeletal collection, and specimen O2.D44, a foetal femur from the University of Otago Anatomy Museum. The results of the age-at-death estimations are presented first, followed by the results of the micro-CT scanning, and the microbial bioerosion assessment.

5.1 Age-at-death estimations

While age-at-death estimations for the ‘Atele skeletal sample have previously been calculated by Pietrusewsky (1969) and (Buckley 2001), objective one of this project was to produce new, independent age-at-death estimations for the eight individuals with previous age-at-death estimations of birth to around one year of age. Dental development standards by Liversidge et al. (1993), Moorrees et al. (1963a), Moorrees et al. (1963b), and Ubelaker (1989) were used in this project in conjunction with diaphyseal length standards produced by Carneiro et al. (2013), Merchant and Ubelaker (1977), and Scheuer et al. (1980). The results of these calculations have been summarised in Table 5.1.

One of the ‘Atele individuals (To-At-2/2) was estimated to have died around 35.8 gestational weeks, placing it within the foetal (eight gestational weeks to birth), stillborn (foetal death after 28 gestational weeks), and perinate (infant born alive from 24 gestational weeks) age categories (Lewis 2007). The age-at-death estimations for the remaining seven individuals suggested that they all died between birth and one year of age, placing them in the infant age category (Lewis 2007). The age-at-death estimations calculated for this project are consistent with those of Buckley (2016) that were used to compile the ‘Atele census.

An age-at-death estimation was also calculated for specimen O2.D44, the foetal femur from the University of Otago W.D. Trotter Anatomy Museum that was selected for this assessment as a ‘control’ sample for a non-burial depositional context. Using the

Carneiro et al. (2013), Merchant and Ubelaker (1977), and Scheuer et al. (1980) diaphyseal aging methods, an age-at-death estimation of *ca.* 22.6 gestational weeks was calculated for this individual (Table 5.1.).

Table 5.1. Summary of the results of the age-at-death estimations.

	Standard						
	Moorrees et al. (1963a, 1963b)	Ubelaker (1989)	Liversidge et al. (1993)	Merchant and Ubelaker (1977)	Scheuer et al. (1980)	Carneiro et al. (2013)	Age-at-death
To-At-1/1b	0.75	0.75	1.0	0.5	N/A	N/A	0.75
To-At-1/5	0.25 – 0.5	0	0.25	0.25	41.3W	38.9W	0 – 0.25
To-At-1/13b	N/A	N/A	N/A	0 – 0.5*	41.8W*	39.7W*	0 – 0.25*
To-At-1/16	N/A	0	0.31	0 – 0.25	39.1W	36.9W	0 – 0.25
To-At-1/29c	0.5	0.5	0.52	0.5	N/A	N/A	0.5
To-At-2/2	N/A	N/A	N/A	<0	37.0W	34.6W	35.8W
To-At-2/14	0.25 – 0.5	0.5	0.31	N/A	N/A	N/A	0.25 – 0.5
To-At-2/17	N/A	N/A	N/A	0.5 – 1.0	58.0W	N/A	0.5 – 1.0
02.D44	N/A	N/A	N/A	<0	23.6W	21.6W	22.6W

Note: all calculations are in years apart from those made using the Scheuer et al. (1980) and Carneiro et al. (2013) standards, which are in weeks (W). Cases where age had to be estimated using only a bone fragment are indicated with an *.

5.2 Micro-CT

Objective two involved using micro-CT imaging to image the internal microstructure of an appropriate bone from each of the infants included in this assessment. The SkyScan 1172 scanner (Bruker-MicroCT, Kontich, Belgium), housed in the Otago Micro and Nanoscale Imaging unit (OMNI) at the University of Otago that was used in this assessment required the objects being scanned to be within 55x55x55mm (OMNI n.d). The fetal femur from the W.D. Trotter Anatomy Museum skeletal collection and eight of the ‘Atele infants had bones that fit these specifications. Test scans were run on the distal ulna fragment of To-At-2/2 and the misc. rib fragment of To-At-2/14, at a resolution of 8.7µm. Assessment of the images captured from these scans showed that microbial bioerosion could be discerned at this resolution (T. Booth,

pers. comm.), so the remaining samples were scanned at the same resolution. A test scan was run on the miscellaneous rib fragment of To-At-1/5 at 5µm, as this was the upper threshold of the resolutions used by Booth et al. (2016). While the level of detail observable increased, it did not increase enough to warrant the time-consuming process of scanning the rest of the samples at the higher resolution. A large volume of images was captured during each scan. The total number of images captured during each scan is summarised in Table 5.2.

5.3 Assessment of microbial bioerosion

For objective three, the micro-CT images captured for the eight 'Atele individuals and the foetal femur from the University of Otago Anatomy Museum were assessed for microbial bioerosion using the Oxford Histological Index (OHI). Multiple images were assessed for each individual beginning with the first image of each series and followed by every 100th image in the series. The final image in the series was also assessed. The number of images assessed for each individual is summarised in Table 5.2. The OHI scores for each image were averaged to produce a single, overall OHI score for each individual. The results of this assessment can also be seen in Table 5.2.

Table 5.2. Summary of the results of the micro-CT scanning and microbial bioerosion assessment.

Infant	Bone scanned	Images capture	Images assessed	Wedl MFD	Non-Wedl MFD	OHI score	Preservation of periosteal surface
To-At-1/1b	Radius fragment	1047	12	No	Yes	0	Yes
To-At-1/5	Misc. rib fragment	1047	12	No	Yes	2	Yes
To-At-1/13b	Misc. rib fragment	1043	12	No	Yes	0/4	Yes
To-At-1/16	Radius fragment	1047	12	No	Yes	0	Yes
To-At-1/29c	Radius fragment	1045	12	No	Yes	0	Yes
To-At-2/2	Ulna fragment	2095	22	No	Yes	2	Yes
To-At-2/14	Misc. rib	2095	22	No	Yes	0	Yes
To-At-2/17	Misc. rib	1047	12	No	Yes	1	Yes
O2D44	Femur	1047	12	No	No	5	Yes

Note: For OHI scores refer to Table 4.3. on page 62.

Non-Wedl MFD was observed in all of the images assessed for the ‘Atele infants. Four of the individuals assessed (To-At-1/1b, To-At-1/16, To-At-1/29c, and To-At-2/14), were given an overall OHI score of zero, with evidence of extensive bioerosion and no identifiable original features in the bone microstructure (Figure 5.1 – 5.4). Of the remaining infants, To-At-2/17 (Figure 5.5.) was given an overall OHI score of one, with small amounts of preserved bone microstructure. To-At-1/5 and To-At-2/2 (Figure 5.6 – 5.7) were given overall OHI scores of two, with some preservation of the bone microstructure between destructive foci. The remaining infant, To-At-1/13b, was given two overall OHI scores due to areas of differing preservation being observed within the first six images assessed, with a score of zero in the superior portion and a score of four in the inferior portion (Figure 5.8). This pattern of preservation was not observed in the remaining six images, with three of these images given scores of zero and the other three given scores of one. Overall, a similar level of variation was seen within and between the different bones scanned, suggesting that location within the skeleton had little impact on preservation. Other than the variation seen in To-At-1/13b, the only other infant with variation in the images assessed was To-At-2/17, but this variation was only by one unit (zero). All of the ‘Atele individuals displayed some preservation of the periosteal surface.

Non-Wedl MFD was not observed in the images assessed for O2.D44, the foetal femur from the Anatomy museum skeletal collection (Figure 5.9). There was no variation in OHI score throughout this series, with all images assessed given an OHI score of five, meaning the microstructure was very well preserved. The periosteal margin was well-preserved in this bone.

5.4 Conclusion

This chapter outlined the results of the age-at-death estimations, micro-CT imaging, and microbial bioerosion assessments performed on the remains of eight infants from the ‘Atele skeletal collection. Seven of the ‘Atele individuals received age-at-death estimations that placed them in the infant age category (birth to one year of age), with the final ‘Atele individual receiving an age-at-death estimation of *ca.* 35.8

gestational weeks, placing it in the foetal, stillborn, and perinate age categories. All these individuals displayed extensive evidence of non-Wedl MFD. A fetal femur (O2.D44) from the W.D. Trotter Anatomy Museum, was also assessed as a control for a non-burial depositional context. This femur was given an age at death estimation of *ca.* 22.6 gestational weeks and had no evidence of non-Wedl.

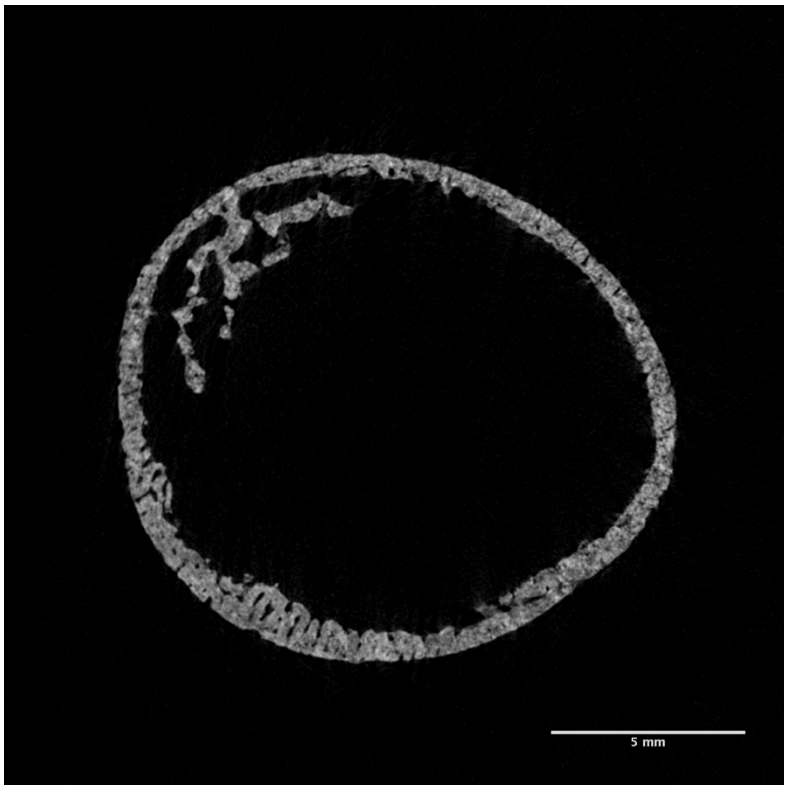

Figure 5.1. Micro-CT image of To-At-1/1b.

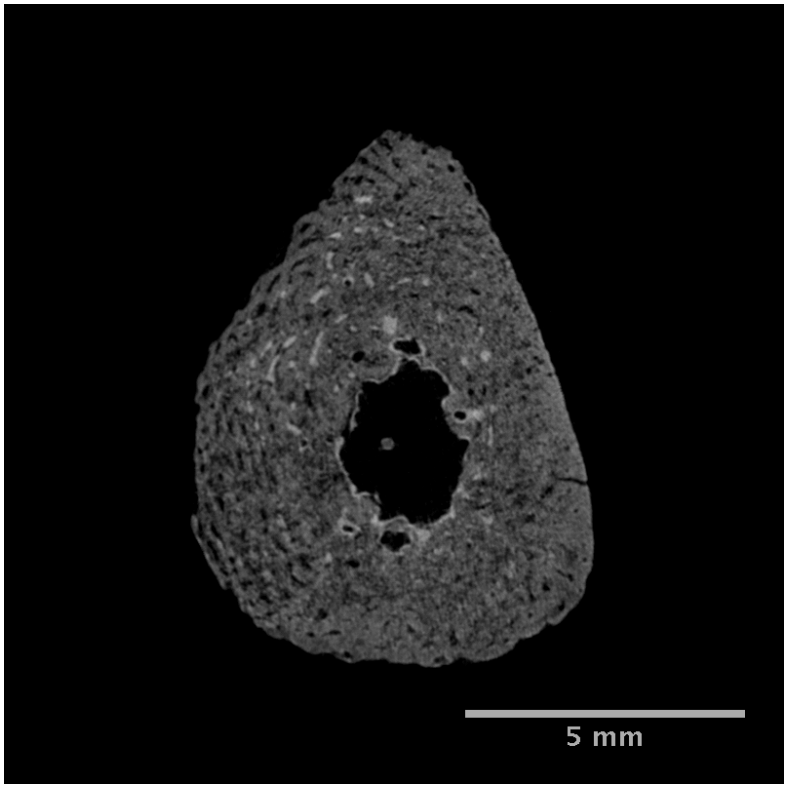

Figure 5.2. Micro-CT image of To-At-1/16.

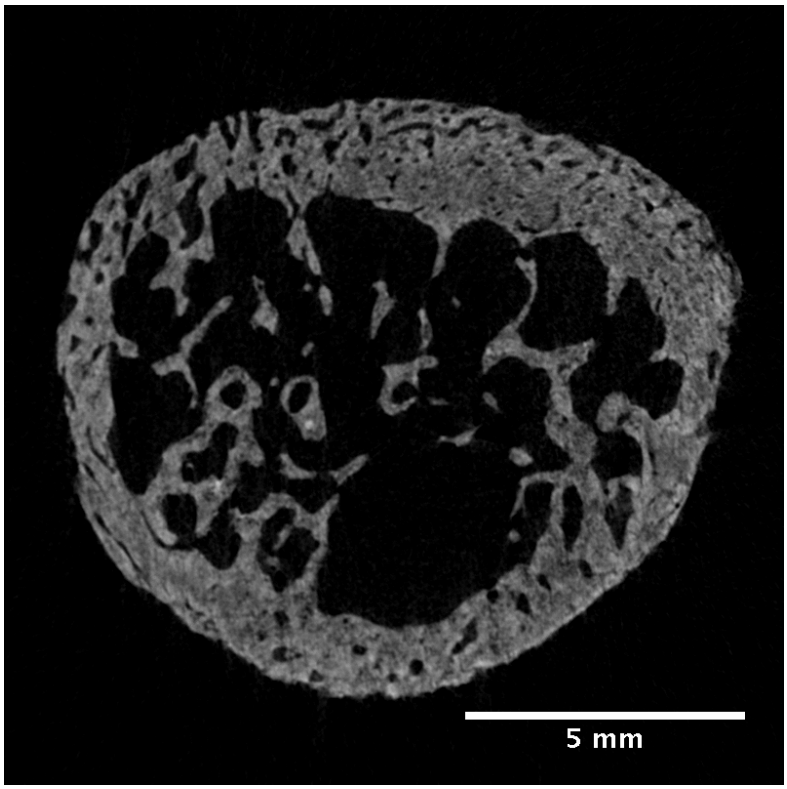

Figure 5.3. Micro-CT image of To-At-1/29c.

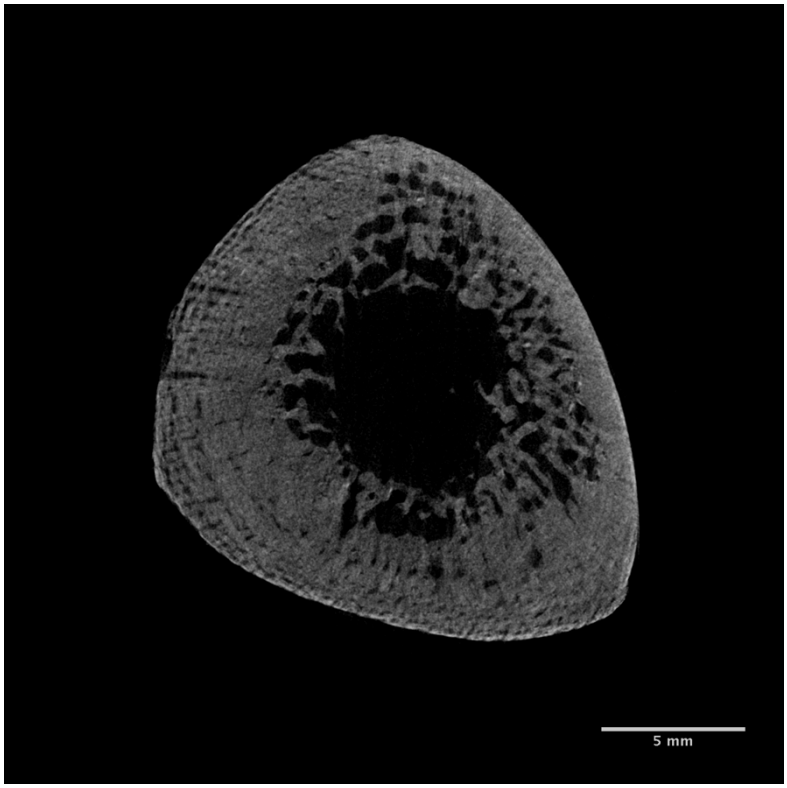

Figure 5.4. Micro-CT image of To-AT-2/14.

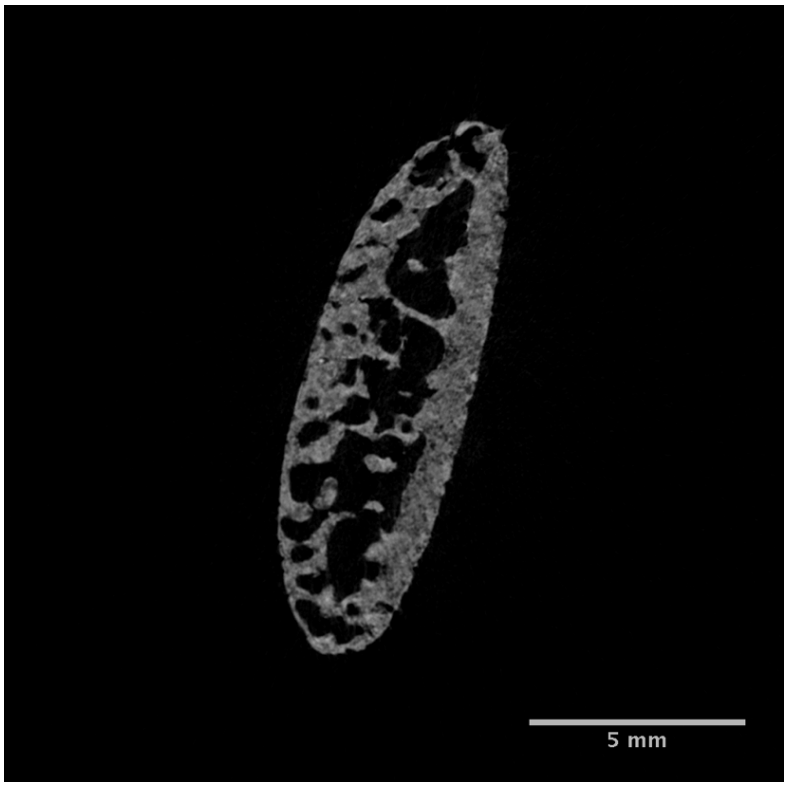

Figure 5.5. Micro-CT image of To-At-2/17.

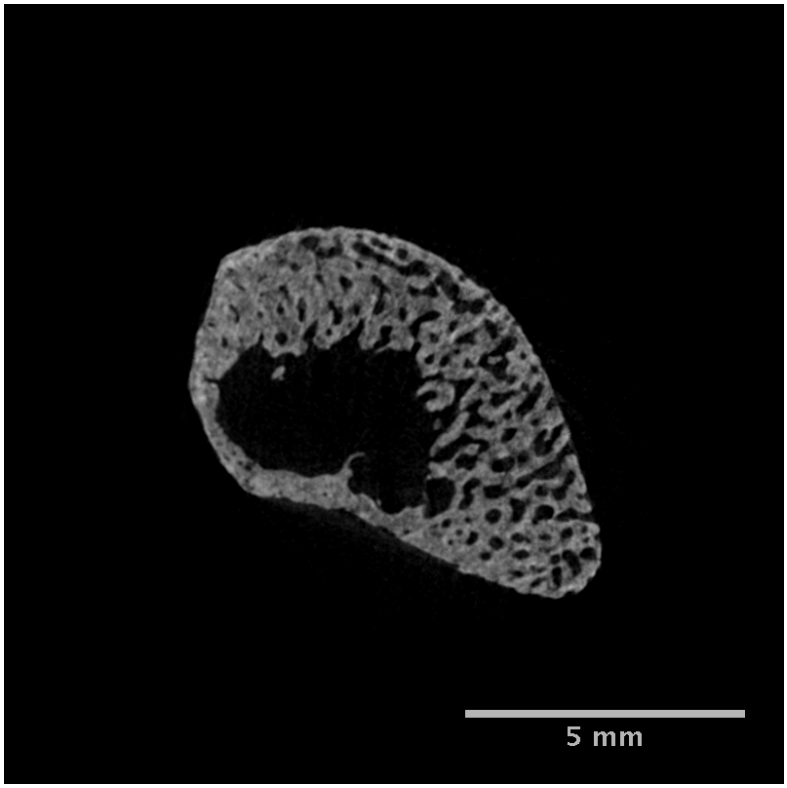

Figure 5.6. Micro-CT image of To-At-1/5.

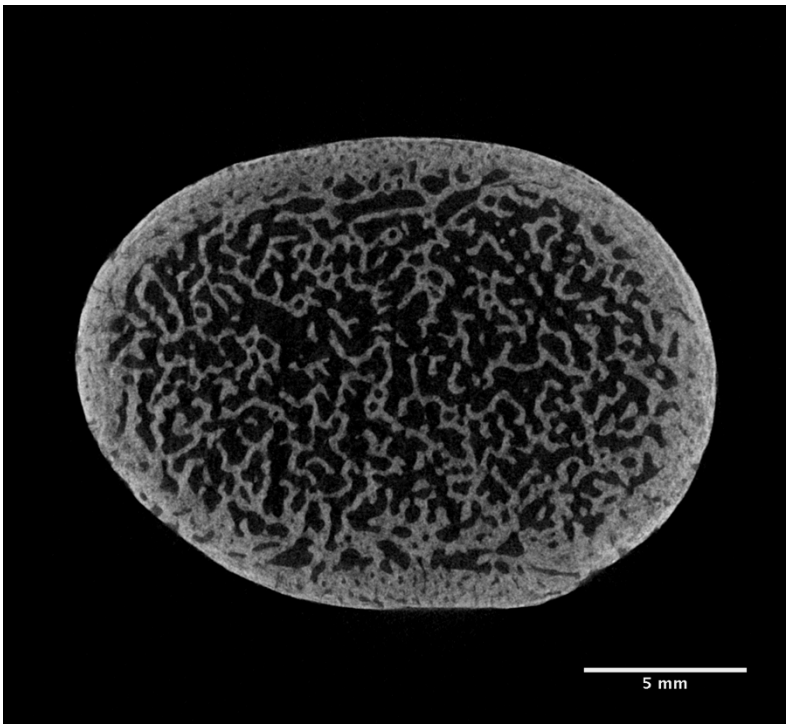

Figure 5.7. Micro-CT image of To-At-2/2.

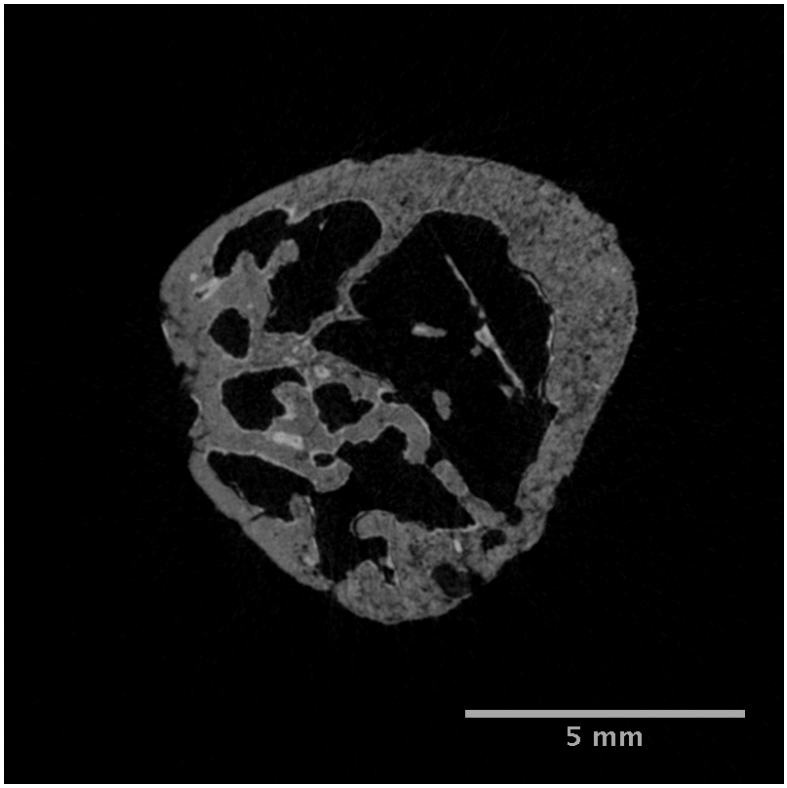

Figure 5.8. Micro-CT image of To-At-1/13b.

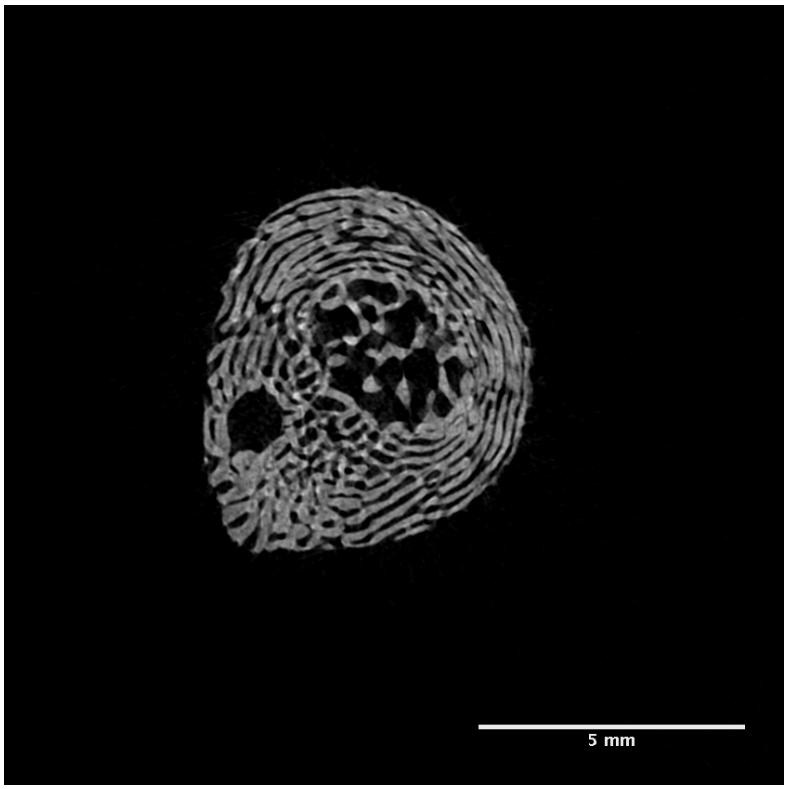

Figure 5.9. Micro-CT image of Anatomy Museum specimen.

6 Discussion

Two aims were addressed in this research. The first aim was to test the efficacy of micro-CT imaging to assessments of microbial bioerosion in archaeological infant skeletal remains from a tropical environment, and the second was to provide insights into the biosocial context of the ‘Atele population within the Chiefdom period of Tongan culture history. This was achieved through four objectives:

5. Firstly, infants from the ‘Atele skeletal collection aged from birth to around one year of age were selected using a census compiled for Buckley (2001). A fetal femur from the University of Otago, W.D. Trotter Anatomy Museum was also selected for this assessment to act as a control. New, independent, age-at-death estimations for these infants were performed as part of this research using dental formation, dental eruption, and diaphyseal length ageing standards. Preference was given to diaphyseal length aging methods.
6. Secondly, micro-CT imaging was used as a non-destructive method to image the internal microstructure of an appropriate bone from each infant.
7. Thirdly, the Oxford Histological Index (OHI) was used to assess the images captured of each bone for microbial bioerosion.
8. And lastly, the results of this assessment were compared with the results of previous research performed on the ‘Atele skeletal collection, particularly that which investigates the health of this population.

The results relevant to these aims and objectives are discussed in this chapter. First, the efficacy of micro-CT to assessments of microbial bioerosion in archaeological infant skeletal remains is discussed. This includes a discussion of micro-CT compared to traditional histological imaging methods. Next, how the results of this project fit within the biosocial context of the chiefdom period of Tongan culture history is discussed. This is followed by a discussion on whether assessments of microbial bioerosion can be used to differentiate perinatal infants from stillborn infants and post-neonatal infants, and if microbial bioerosion can be used as a feeding signal. This closes with some concluding remarks.

6.1 The efficacy of micro-CT to assessments of microbial bioerosion in archaeological infant skeletal remains.

As discussed in Chapter 4.2, micro-CT imaging was chosen for this assessment because it provides a non-destructive method to capture high resolution images of the internal microstructure of bone (Booth 2020; Booth et al. 2016; Rühli et al. 2007). Eight 'Atele infants and a 'control' foetal femur from the W.D. Trotter Anatomy Museum were micro-CT imaged at a resolution of 8.7µm, before being assessed for bioerosion using the Oxford Histological Index. As discussed in Chapter 4.3, the Oxford Histological Index (OHI) was developed by Hedges et al. (1995) and Millard (2001) to describe the percentage of unaltered bone microstructure in a section of bone. It does so by employing a zero to five scoring system where a score of zero means that no original features other than Haversian canals are identifiable, and a score of five means that the microstructure of the section is virtually indistinguishable from that of fresh bone (Hedges et al. 1995; Millard 2001).

Non-Wedl microscopical focal destruction (MFD), which is formed when bacteria breakdown the mineral component of bone to access bone collagen, was observed in all of the 'Atele infant skeletal remains assessed. Non-Wedl MFD was not observed in the 'control' foetal femur from the W.D. Trotter Anatomy Museum collection. The lack of non-Wedl bioerosion in this bone is consistent with previous research by Jans et al. (2002), White and Booth (2014), Booth et al. (2016), and Booth (2016), where foetal and stillborn infant skeletal remains lacked evidence of non-Wedl MFD. This is believed to be related to the relative sterility of the foetal gastro-intestinal track (Booth 2016; Booth 2020; Booth et al. 2016; White and Booth 2014). The lack of non-Wedl MFD was also expected because the non-burial depositional context of this bone means that it was not exposed to environmental soil microbes.

As for infant To-At-2/2, which was given an age-at-death estimation of *ca*. 35.8 gestational weeks, non-Wedl MFD was observed in all the micro-CT images assessed. This age-at-death estimation suggests To-At-2/2 could have been a stillborn infant, or could have died during the perinatal period, having been a premature birth or a small for gestational age infant. The presence of non-Wedl MFD in the micro-CT images assessed suggests To-At-2/2 may have survived long enough after birth for the gut

microflora to form before dying during the perinatal period. It is also possible that To-At-2/2 was a small for gestational age (SGA) infant. This is difficult to test as only skeletal material was recovered for this individual, making dental age-at-death estimations unavailable for comparison. Discrepancies between these ages can suggest that an infant was SGA. The use of skeletal ageing methods alone can under-age SGA infants, placing them within the foetal and stillborn age categories (Lewis 2007). SGA infants are an important consideration when investigating the health of past populations because a number of issues including maternal ill-health and malnutrition, can cause growth restriction (Lewis 2007; Owsley and Jantz 1985).

Another possibility for the observation of non-Wedl MFD in the micro-CT images captured for To-At-2/2 is that other taphonomic processes were acting to decrease the preservation of the internal microstructure of the bone. As discussed in Chapter 2.2.3, the movement of water through burial environments has an immense impact on the preservation of bone, leaching it of its mineral content and increasing the size of the natural porosities in its microstructure (Hedges and Millard 1995; Kendall et al. 2018; Turner-Walker 2008). This in turn, increases the ability of microbes to enter bone from the burial environment to produce bioerosion. For both of the ‘Atele burial mounds, burial pits were dug into the existing soil surface and then partially filled in with white sand (Davidson 1969). The use of white sand in the burials and the repetitive opening of the mounds for further burials, led to significant mixture of the soil with the sand (Davidson 1969). The sandy matrix of the ‘Atele burial mounds would allow water to move freely through the burials, so it is possible that mineral leaching allowed bacteria from the burial environment to move in and produce non-Wedl MFD even if To-At-2/2 did not survive long enough for the gut microflora to form post-natally. This is difficult to test in the absence of other potential foetal-aged individuals. The ‘Atele skeletal collection also has good macroscopic preservation, suggesting that environmental taphonomic processes were not greatly affecting preservation.

In regards to variation in OHI scores throughout all the images assessed for each individual, four infants displayed variation: To-At-1/13b, To-At-1/16, To-At-2/2, and To-At-2/17. An interesting result was seen in the miscellaneous rib fragment of infant To-At-1/13b. The first six micro-CT images were given two overall OHI scores, a score

of one in the anterior portion of the bone and a score of four in the more posterior portion of the bone. This result could reflect the subjective nature of the OHI. Research has also shown preservation of skeletal material to vary not just between skeletons excavated from the same site, but also within individual skeletons and individual bones (Booth et al. 2016; Hanson and Buikstra 1987; Hedges 2002). It is possible then, that OHI scores could vary within a bone in the manner in which they varied within the miscellaneous rib fragment of To-At-1/13b. To-At-2/2 displayed the greatest range of OHI scores, being assigned scores ranging from zero to four. This variation is most likely related to the difficulty experienced when trying to identify bioerosion in the micro-CT images captured for this individual. In general, difficulty was found in applying the OHI to the immature woven bone studied in this assessment given that the OHI is designed more for mature lamellar bone. As discussed in Chapter 2.1.2., bone starts out as woven bone which is characterised by a disorganised, non-uniform distribution of collagen fibers and cells (Steiniche and Hauge 2004; Stevens and Lowe 2005; Walsh et al. 2004). Over time, woven bone is replaced by lamellar bone which is characterised by an organised structure of collagen fibers in parallel sheets (lamellae) that are arranged around Haversian canals (Steiniche and Hauge 2004; Stevens and Lowe 2005; Walsh et al. 2004; White et al. 2012). Not only do the descriptions provided for each score on the OHI relate heavily to the relationship of bioerosion to lamellae and Haversian systems, so to do the morphological descriptions developed to identify it. The lack of Haversian systems in infant woven bone made it difficult to identify bioerosion in some of the infants assessed, and to assign OHI scores. In this assessment, non-Wedl MFD was identified where the contrast between areas of low and high density caused the woven bone within the samples to become “mottled” in appearance, and when the periosteal surface was preserved. This criterion was based on patterns noted in similar studies, particularly Booth et al. (2016). Given the increase in interest regarding bioerosion and infant skeletal remains, it would be beneficial to future research if a method for the identification and quantification of bioerosion specific to infant woven bone was developed. Reference images for each level of the OHI scoring system would be particularly useful.

Similar OHI scores were seen between the different types of bones scanned in this project, suggesting that location within the skeleton had little impact on

preservation. It is possible however, that this result is due to only a single bone from each individual being scanned in this project. For future research investigating whether location within the skeleton impacts the development of non-Wedl MFD, it would be advantageous to scan multiple bones from the same individual given that preservation of bone has been shown to be highly variable (Booth et al. 2016; Hanson and Buikstra 1987; Hedges 2002).

6.1.1 Micro-CT imaging compared to traditional histology techniques

Overall, the results of this study show that micro-CT imaging can be used to image the internal microstructure of bone for assessments of microbial bioerosion. It would be useful to compare the micro-CT images produced in this study to images produced using traditional histology techniques. One reason for this is that while a main advantages of micro-CT is that it is non-destructive and captures a large volume of data, higher resolutions are achievable using histological techniques (Cooper et al. 2012).

Booth et al. (2016) found comparable OHI scores when assessing micro-CT images captured of mid-shaft femoral fragments from the three adult individuals that had been previously excised and assessed for bioerosion using transmitted light microscopy. Based on this, it is most likely that comparable results would be seen for the ‘Atele infants if they were assessed for microbial bioerosion using traditional histology imaging techniques.

As discussed in Chapter 4.2.1, two of the key advantages of micro-CT is that it is a non-destructive and produces a larger volume of images than traditional histological techniques. Rühli et al. (2007) compared the diagnostic value of micro-CT imaging to well established histology methods using a sample of autopsy-based macerated human skulls with macroscopic evidence of pathological change. This study found that while pathological changes to the microstructure of the bone could be observed using micro-CT imaging, some bony responses could only be observed using histology methods (Rühli et al. 2007). The large volume of images captured using micro-CT was seen as the main advantage of Micro-CT over histology methods (Rühli et al. 2007). This is because it is not always easy to excise the correct portion of bone for histological

examination as some pathological changes are not always visible macroscopically (Rühli et al. 2007). The variation in the OHI scores observed in this assessment also highlights the advantage of the volume of images captured using Micro-CT.

Dal Sasso et al. (2014) assessed 58 samples of human cortical bone from individuals excavated from Al Khiday in Sudan for microbial bioerosion using both micro-CT imaging and scanning electron microscopy (SEM). While microbial bioerosion could be identified in the micro-CT images captured, the limitations of the spatial resolutions achievable (3 - 5µm) meant that single pores and thin micro-tunneling smaller than 2µm could not be identified (Dal Sasso et al. 2014). The 8.7µm spatial resolution achieved in this study meant that non-Wedl MFD could be identified but could not be further categorised as linear-longitudinal, lamellate, or budded non-Wedl MFD. While it was not necessary for this project to identify these subcategories of non-Wedl MFD, it highlights one of the advantages of traditional histology methods over micro-CT: the spatial resolutions achievable. Comparable spatial resolutions are achievable by both histology and micro-CT, however the resolutions achievable with micro-CT are limited by the parameters of the scanner used and the size of the sample being scanned. As mentioned above, difficulty was met when applying the OHI to the infant woven bone assessed due to the lack of identifiable structures in the bone microstructure. It is possible that at higher resolutions these structures would be easier to identify making assessments of microbial bioerosion easier. A test scan was run at a resolution of 5µm but this did not significantly increase the quality of the information captured. Another disadvantage of the size requirements of micro-CT is that adult bone can only be scanned if sections are excised or if fragments are targeted (Booth et al. 2016).

6.2 How the results of this project fit within the biosocial context of the Chiefdom period of Tongan culture history.

The second aim of this research is to provide insights into the biosocial context of the ‘Atele population within the Chiefdom period of Tongan culture history. This is done in the following section by comparing the results of this assessment to the results of previous research performed on the ‘Atele population, particularly that which

investigated the health of this population. It is important to discuss the results of this assessment within the biosocial context of the Chiefdom Period because, as discussed in Chapter 1, infant health can have serious implications for maternal health, population health, and the adaptive success of populations (Goodman and Armelagos 1989; Lewis 2007).

All but one of the ‘Atele individuals assessed in this project had age-at-death estimations that placed them within the infant age category. The final individual was To-At-2/2, which as discussed in Chapter 5.1, was given an age-at-death estimation of *ca.* 35.8 gestational weeks placing it within the foetal, stillborn, and perinatal age categories. The observation of non-Wedl MFD in the images captured for this individual means that To-At-2/2 likely survived long enough after birth for the gut microflora to form before dying during the perinatal period. Three of the older infants had age-at-death estimations of 0 – 0.25 years, meaning that they could have also died within the perinatal period. These individuals could have also died within the neonatal period, which is defined as from birth to 27 postnatal days (Lewis 2007). Following the birthing process, the perinatal and neonatal periods are considered the most critical times in a newborn’s life (Halcrow and Tayles 2011; Halcrow et al. 2017). Infant mortality during this time is known as endogenous mortality as it is thought to be related to endogenous issues including congenital problems, prematurity, low birth weight, and birth trauma (Kinaston et al. 2009; Lewis and Gowland 2007; Scott and Duncan 1999:41). Infant mortality after this period has been related to exogenous issues including environmental insults, infectious disease, malnutrition, poisonings and accidents (Scott and Duncan 1999:41-42). Maternal health plays a significant role in infant health and endogenous mortality. As discussed in Chapter 1.1, foetuses are fully dependent on their mothers for nutrition and protection from environmental insults, so foetal health is indirectly influenced by maternal health (Goodman and Armelagos 1989; Lewis 2007). Even after birth, maternal health impacts infant health through the passive immunity provided by breast milk (Goodman and Armelagos 1989; Lewis 2007). As maternal health is determined by exogenous socio-economic, cultural, nutritional, and health related factors specific to the population the mothers come from, infant health and mortality can also act as an indicator of population health (Lewis 2007). Infant health is also an important indicator of population health as infant ill-

health has been shown to have lasting, life-long impacts on health (Barker 2012; Gowland 2020; Gowland 2015). The "First 1000 days after conception" in particular, are considered to have a crucial impact on life-long health (Barker 2012:186). For example, low birth weight has been linked to coronary heart disease, type two diabetes, and hypertension (Barker et al. 2002).

Buckley (2000; 2001; 2016) investigated the health of the 'Atele population by examining skeletal and dental evidence of ill-health. Non-specific skeletal stress indicators were reported within the 'Atele skeletal collection by Buckley (2000) and Buckley (2016). Health concerns such as iron-deficiency anaemia, haematogenous osteomyelitis, congenital syphilis, yaws, scurvy, hypervitamintosis A, trauma, and Caffey's disease, could have caused these lesions (Buckley 2000:481). The presence of linear enamel hypoplasia (LEH), a hypoplastic developmental defect of enamel (DDE) that forms when matrix secretion is disturbed during enamel formation, on the teeth of some of the 'Atele individuals suggests that they were experiencing stress episodes significant enough to cause growth disturbance (Buckley 2016). The long period of time that dental formation covers, from the prenatal period through childhood, makes assessments of DDE excellent measures of population health (Hillson 1996). In addition, teeth do not remodel so any stress episodes significant enough to cause growth disturbance can leave a permanent marker on the dentition (Hillson 1996). A comprehensive analysis of all DDE present in the dentition of the 'Atele skeletal collection was performed by Barker (2016) to explore evidence of early life stress within the 'Atele population. An interesting result from this analysis was that the females in the 'Atele skeletal collection had a significantly higher prevalence of DDE than men in both mounds (Barker 2016). This suggests that the females within the population could have been experiencing more stress episodes than their male counterparts. This result is consistent with the isotope analysis performed by Stantis (2015), who found significantly lower δ^{15} nitrogen values in the females of the 'Atele skeletal collection. Nitrogen stable isotope values are used to infer the trophic level and level of animal protein in an individual's diet (Stantis 2015; Stantis et al. 2015). It is possible that the lower δ^{15} nitrogen values of the females of the 'Atele skeletal collection means that they were consuming less animal protein than the males, perhaps

due to cultural restrictions (Stantis 2015). Protein is a key nutritional requirement so restrictions on its consumption could have had serious repercussions on the health of these individuals, evidenced by the increased prevalence of DDE (Barker 2016; Jelliffe 1967; Stantis 2015). If restrictions on the consumption of animal protein had negative repercussions on female health and as an extension, maternal health, the large proportion of early infancy deaths in this population could indicate that it also had negative repercussions for foetal and infant health.

6.3 Can assessments of microbial bioerosion be used to stillborn infants from older post-natal infants?

Because foetal and infant mortality can be an important indicator of not just maternal and population health, but also of the adaptive success of a population, it is important to have accurate age-at-death estimations for stillborn, perinatal, and neonatal infants. As discussed in Chapter 4.1, numerous dental and skeletal standards for the estimation of infant age-at-death have been developed (Carneiro et al. 2013; Fazekas and Kósa 1978; Liversidge et al. 1993; Merchant and Ubelaker 1977; Moorrees et al. 1963a; Moorrees et al. 1963b; Scheuer et al. 1980; Ubelaker 1989). A number of issues must be considered when using these standards. One of these issues is the inherent variability of physiological age-at-death from chronological age-at-death due to the lack of written death records for archaeological populations (Lampl and Johnston 1996). This variation further impacts the accuracy of age-at-death estimations because populations where chronological age-at-death is known are often used to develop the ageing standards applied to archaeological samples (Lampl and Johnston 1996). These standards are specific to the populations used to develop them, populations that are often of a vastly different environmental, socio-economic, and chronological contexts to the archaeological populations they are applied to (Lampl and Johnston 1996). This is particularly an issue for the ‘Atele skeletal collection as there are no aging standards available that have been developed using Pacific Island samples, and research has demonstrated advanced dental formation and eruption in Pacific Island children (Fry 1976; Te Moananui et al. 2008). Some of these issues can be avoided by using a combination of dental and skeletal aging methods, because as discussed above,

discrepancies in these ages can indicate prematurity and growth restriction (Lewis 2007; Owsley and Jantz 1985). There were no significant differences in the skeletal and dental age-at-death estimations calculated in this study.

Overall, these issues with aging standards make it difficult to differentiate stillborn infants from older post-natal infants (Lewis 2007; Lewis and Gowland 2007). If it is true that infants who do not survive long enough for the gut microflora to form do not display evidence of bacterial bioerosion, then assessments of microbial bioerosion could be used in conjunction with ageing methods to help differentiate these individuals. This is supported by the observation of non-Wedl MFD in the micro-CT images captured for infant To-At-2/2, which as discussed above, suggests this individual may have been born alive and survived long enough for the gut microflora to form before dying during the perinatal period. The results of this assessment show the potential for assessments of microbial bioerosion to be used to differentiate stillborn and older post-natal infants. To test this further, assessments of microbial bioerosion should be performed on larger samples of infants. While the 'Atele skeletal collection has one of the largest collections of infants from a Pacific Island archaeological context, the sample size of this project (n=8) was too small for statistical analysis. The potential of assessments of microbial bioerosion to differentiate stillborn and older post-natal infants was also investigated by Booth et al. (2016). As discussed in Chapter 2.3.2, Booth et al. (2016) examined ten perinatal infants for non-Wedl MFD. Of these perinates, four had age-at-death estimations that suggested they were preterm, and the remainder were older (Booth et al. 2016). Of the four preterm individuals, three lacked evidence of non-Wedl MFD (Booth et al. 2016). This sample also deemed too small for statistical analysis.

6.3.1 Non-Wedl bioerosion as a feeding signal

One way in which assessments of microbial bioerosion could further help differentiate stillborn infants from older post-natal infants is if observations of microbial bioerosion could be used as a feeding signal. As discussed in Chapter 2.3.2, the relative sterility of foetal gastro-intestinal tracks is thought to be rapidly lost around birth (Brooks et al. 2014; Groer et al. 2014; Mackie et al. 1999; Penders et al. 2006). A major way in which the gastro-intestinal tract is rapidly colonised is through feeding practices,

so observations of microbial bioerosion in the bones of short-lived infants such as To-At-2/2, could provide a signal that an infant survived long enough after birth for at least their first feed. This could be through breastfeeding or from being hand-fed milk substitutes (Knodel and Kintner 1977).

Feeding practices have been shown to play a significant role in infant mortality, particularly whether and infant was breastfed. Breastmilk represents a nutritionally ideal and clean food source for newborn infants, as well as providing some passive immunity from disease (Goodman and Armelagos 1989; Knodel and Kintner 1977; Lewis 2007). Knodel and Kintner (1977), observed variations in perinatal and neonatal mortality in populations where breastfeeding was common and those where it was not. In populations where breastfeeding was uncommon or of short duration and where infants were artificially hand fed, perinatal and neonatal mortality was high (Knodel and Kintner 1977). Low levels of perinatal and neonatal mortality were observed in populations where breastfeeding was common (Knodel and Kintner 1977). In these populations, infant mortality levels remained low until weaning ages (Knodel and Kintner 1977).

Some researchers have used stable isotope analyses to investigate breastfeeding signals (Fogel et al. 1997; Fogel et al. 1989; Fuller et al. 2006a; Fuller et al. 2006b; Kinaston et al. 2009; Nitsch et al. 2011; Siebke et al. 2019). When nursing, infants are essentially consuming their mothers' tissue through breast milk and are considered to be one trophic level above their mothers (Fuller et al. 2006b; Kinaston et al. 2009). This results in a 2-3‰ increase in the δ^{15}nitrogen values of infant bone collagen compared to that of adult females, and this has been interpreted as a breastfeeding signal (Fogel et al. 1997; Fogel et al. 1989; Fuller et al. 2006b; Kinaston et al. 2009). In most cases, changes in δ^{15}nitrogen values have also been used to investigate weaning practices in archaeological populations because the elevated δ^{15}nitrogen values seen with breastfeeding decrease when breastfeeding ends and supplementary weaning foods are introduced (Fogel et al. 1997; Fogel et al. 1989; Fuller et al. 2006b; Katzenberg 2008; Kinaston et al. 2009).

There are a number of issues that need to be considered when using elevated δ^{15}nitrogen values in infants as a breastfeeding signal. Ideally, the female δ^{15}nitrogen values used as a comparison would come from the mother of that infant, however, other than in rare cases such as coffin births, the nature of archaeological samples makes it impossible to know for sure if female remains associated with infant remains are related (Siebke et al. 2019). Instead, average δ^{15}nitrogen values of all the females within a sample are often used for comparison (Siebke et al. 2019). This can introduce error as women who are pregnant have been shown to have different isotope ratios to women who are not pregnant (Fuller et al. 2004; Siebke et al. 2019). Another issue is that elevated δ^{15}nitrogen values in infants can occur for a number of other reasons, including poor maternal health, nutritional deficiency, and because of metabolic disorders suffered by the mother or child (Kinaston et al. 2009; Siebke et al. 2019). Lastly, perinatal and neonatal infants may die too early for their bones to metabolise N^{15}-enriched breastmilk (Katzenberg 2008; Kinaston et al. 2009; Stantis 2015). Because of issues like these, it is recommended that stable isotope analysis should only be used in conjunction with other methods to investigate the timing of breastfeeding (Siebke et al. 2019). Assessments of microbial bioerosion could be one of those methods.

Stantis (2015) used stable isotope analysis to investigate breastfeeding and weaning practices in the ‘Atele population. The δ^{15}nitrogen values of all but two of the ‘Atele infants examined were elevated, suggesting that they were breastfeed (Stantis 2015). The remaining two infants displayed noticeably lower δ^{15}nitrogen values, well within the mean of the adult females examined (Stantis 2015). Given that these infants were aged 0 and 0.25, it was suggested that these infants had died before their bones could metabolise ^{15}N enriched breastmilk. As the age-at-death estimations used in this study were those calculated for Buckley (2001), it is likely that the infant aged 0 was To-At-2/2, the only infant given that age-at-death estimation. While elevated δ^{15}nitrogen values were not observed for this individual, the observation of non-Wedl MFD in the micro-CT images assessed for this individual suggests that they may have survived long enough after birth for their GIT to be colonised through feeding. When using the presence of non-Wedl MFD in perinatal remains as a feeding signal, it must be acknowledged that recent research has shown potential for a prenatal microbiome to

exist, with bacteria observed in the cord blood and meconium of healthy neonatal infants (Ardissone et al. 2014; Booth 2020; Ferretti et al. 2018; Groer et al. 2014; Jiménez et al. 2005; Jiménez et al. 2008; Matamoros et al. 2013; Mueller et al. 2015).

6.4 Conclusion

The first aim of this thesis was to test the efficacy of micro-CT imaging to assessments of microbial bioerosion in archaeological infant skeletal remains from a tropical environment. The infants (n=8) studied in this assessment were excavated from two burial mounds in the ‘Atele region of Tongatapu Island (Davidson 1969). Microbial bioerosion, in the form of bacterially mediated non-Wedl microscopical focal destruction (MFD), was observed in all the micro-CT images assessed for these infants. This suggests that micro-CT is an appropriate, non-destructive imaging method for assessments of microbial bioerosion. However, a certain degree of skill and experience is recommended to accurately interpret the information captured. This is particularly important when applying methods such as the Oxford Histological Index to images of immature woven bone, when it was developed for mature lamellar bone.

The second aim of this thesis was to provide insights into the biosocial context of the ‘Atele population within the Chiefdom period of Tongan culture history. This was done by comparing the results of this assessment to the results of previous research performed on the ‘Atele population, particularly that which investigated the health of this population. The high level of perinatal and neonatal mortality within the sample assessed reflects previous research performed on the ‘Atele population that suggests female health (and as an extension, maternal health) may have been negatively impacted by cultural restrictions on the level of animal protein in their diet (Barker 2016; Stantis 2015). As discussed above, the perinatal and neonatal periods are considered one of the most critical periods of a newborn’s life, with mortality during this period thought to result from endogenous factors relating to maternal health (Halcrow et al. 2017; Halcrow et al. 2008). Because maternal health is determined by exogenous socio-economic, cultural, nutritional, and health related factors specific to the population the mothers come from, perinatal mortality is also considered a valuable indicator of population health (Lewis 2007). Not only this, but because an infant’s survival is

dependent on their parents and wider community providing them with the means to survive, perinatal mortality can also be considered a valuable indicator of a population's adaptive success (Lewis 2007). It is evident from the high level of perinatal death in the sample of infants assessed that maternal health was being negatively impacted by some factor, likely a combination of nutritional and health stress.

Because of the wealth of information infant skeletal remains can provide, it is important for age-at-death estimations to be as accurate as possible. This is particularly important given the relationship observed between non-Wedl MFD and age-at-death, with significantly lower prevalence rates for non-Wedl MFD being reported for both human and animal stillborn and short-lived (perinate and neonate) infants (Booth 2016; Booth et al. 2016; Jans et al. 2002; White and Booth 2014). This has been linked to the relative sterility of foetal gastro-intestinal tracts, something that is lost rapidly at birth (Booth 2016; Booth et al. 2016; Brooks et al. 2014; Groer et al. 2014; Mackie et al. 1999; Penders et al. 2006; White and Booth 2014). Interestingly, non-Wedl MFD was observed in all the images captured and assessed infant To-At-2/2, who was given an age-at-death estimation of *ca.* 35.8 gestational weeks. It is possible then, that To-At-2/2 could have survived long enough after birth for the gut microflora to form before dying during the perinatal period. This highlights the potential of microbial bioerosion assessments to assist in the differentiation of stillborn infants from older post-natal infants. Because the gut microbiome is rapidly colonised around birth, particularly through feeding, the presence of non-Wedl MFD in the images captured for To-At-2/2, could suggest that this infant survived long enough for at least their first feed, highlighting the potential for microbial bioerosion to also act as a feeding signal.

Overall, this assessment has shown micro-CT to be a valuable non-destructive imaging technique for assessments of microbial bioerosion in archaeological infant remains. It has shown valuable potential for assessments of microbial bioerosion to be used to differentiate stillborn infants from older post-natal infants, as well as potential for its use as a feeding signal.

References

Alexandersen V, Norén JG, Hoyer I, Dietz W, and Johansson G. 1998. Aspects of teeth from archaelogic sites in Sweden and Denmark. Acta odontologica Scandinavica 56(1):14-19.

AlQahtani SJ, Hector MP, and Liversidge HM. 2014. Accuracy of dental age estimation charts: Schour and Massler, Ubelaker and the London Atlas. American Journal of Physical Anthropology 154(1):70-78.

Ardissone AN, de la Cruz DM, Davis-Richardson AG, Rechcigl KT, Li N, Drew JC, Murgas-Torrazza R, Sharma R, Hudak ML, Triplett EW et al. . 2014. Meconium Microbiome Analysis Identifies Bacteria Correlated with Premature Birth. PLoS ONE 9(3):1-8.

Bäckhed F, Roswall J, Peng Y, Feng Q, Jia H, Kovatcheva-Datchary P, Li Y, Xia Y, Xie H, Zhong H et al. . 2015. Dynamics and Stabilization of the Human Gut Microbiome during the First Year of Life. Cell Host & Microbe 17(5):690-703.

Bada JL, Herrmann B, Payan IL, and Man EH. 1989. Amino acid racemization in bone and the boiling of the German Emperor Lothar I. Applied Geochemistry 4(3):325-327.

Barker ACL. 2016. Stress Near the Start of Life: Quantifying the dental enamel defects present in a Chiefdom period sample from Tonga: A dissertation submitted for the degree of Bachelor of Arts with Honours. Dunedin, New Zealand: University of Otago.

Barker DJP. 2012. Developmental origins of chronic disease. Public Health 126(3):185-189.

Barker DJP, Eriksson JG, Forsn T, and Osmond C. 2002. Fetal origins of adult disease: strength of effects and biological basis. International Journal of Epidemiology 31(6):1235-1239.

Behrensmeyer AK, and Kidwell SM. 1985. Taphonomy's Contributions to Paleobiology. Paleobiology 11(1):105-119.

Bell LS. 1990. Palaeopathology and diagenesis: an SEM evaluation of structural changes using backscattered electron imaging. Journal of Archaeological Science 17(1):85-102.

Bell LS, Boyde A, and Jones SJ. 1991. Diagenetic alteration to teeth in situ illustrated by backscattered electron imaging. Scanning 13(2):173-183.

Bell LS, Skinner MF, and Jones SJ. 1996. The speed of post mortem change to the human skeleton and its taphonomic significance. Forensic Science International 82(2):129-140.

Berna F, Matthews A, and Weiner S. 2004. Solubilities of bone mineral from archaeological sites: the recrystallization window. Journal of Archaeological Science 31(7):867-882.

Bethell PH, and Carver MOH. 1987. Detection and enhancement of decayed inhumations at Sutton Hoo. Death, decay and reconstruction. Manchester, UK: Manchester University Press. p 10-21.

Bocquet-Appel J-P, and Masset C. 1982. Farewell to paleodemography. Journal of Human Evolution 11(4):321-333.

Booth T. 2014. An investigation into the relationship between bone diagenesis and funerary treatment: a thesis submitted for the degree of Doctor of Philosophy. Sheffield, UK: University of Sheffield.
Booth T. 2017. The Rot Sets In: Low-Powered Microscopic Investigation of Taphonomic Changes to Bone Microstructure and its Application to Funerary Contexts. In: Errickson D, and Thompson T, editors. Human Remains: Another Dimension. London, England: Academic Press. p 7-28.
Booth TJ. 2016. An Investigation Into the Relationship Between Funerary Treatment and Bacterial Bioerosion in European Archaeological Human Bone. Archaeometry 58(3):484-499.
Booth TJ. 2020. Using Bone Histology to Identify Stillborn Infants in the Archaeological Record. In: Gowland R, and Halcrow SE, editors. The Mother-Infant Nexus in Anthropology: Small beginnings, significant outcomes. Cham, Switzerland: Springer. p 193-209.
Booth TJ, Redfern RC, and Gowland RL. 2016. Immaculate conceptions: Micro-CT analysis of diagenesis in Romano-British infant skeletons. Journal of Archaeological Science 74:124-134.
Boskey AL, Wright TM, and Blank RD. 1999. Collagen and Bone Strength. Journal of Bone and Mineral Research 14(3):330-335.
Bott E. 1982. Tongan society at the time of Captain Cook's visits : discussions with Her Majesty Queen Sālote Tupou. Wellington, New Zealand: Polynesian Society.
Brooks B, Firek BA, Miller CS, Sharon I, Thomas BC, Baker R, Morowitz MJ, and Banfield JF. 2014. Microbes in the neonatal intensive care unit resemble those found in the gut of premature infants. Microbiome 2(1):1-16.
Bruker. 2019. microCT Microtomography.
Buckley HR. 2000. Subadult health and disease in prehistoric Tonga, Polynesia. American Journal of Physical Anthropology 113(4):481-505.
Buckley HR. 2001. Health and disease in the prehistoric Pacific Islands: a thesis submitted for the degree of Doctor of Philosophy. Dunedin, New Zealand: University of Otago.
Buckley HR. 2006. 'The predators within': Investigating the relationship between malaria and health in the prehistoric Pacific Islands. In: Oxenham MF, and Tayles N, editors. Bioarchaeology of Southeast Asia. Cambridge; New York: Cambridge University Press. p 309-332.
Buckley HR. 2016. Health and disease in the prehistoric Pacific Islands. United Kingdom: British Archaeological Reports Ltd.
Buikstra JE, and Ubelaker DH. 1994. Standards for data collection from human skeletal remains : proceedings of a seminar at the Field Museum of Natural History, organized by Jonathan Haas. Fayetteville, Ark.: Arkansas Archeological Survey.
Burley D. 1998. Tongan Archaeology and the Tongan Past, 2850–150 B.P. Journal of World Prehistory 12(3):337-392.
Burley D, Edinborough K, Weisler M, and Zhao J-X. 2015. Bayesian Modeling and Chronological Precision for Polynesian Settlement of Tonga. PLoS One 10(3):1-14.
Burley D, Weisler MI, and Zhao J-x. 2012. High Precision U/Th Dating of First Polynesian Settlement (Dating First Polynesian Settlement). 7(11):1-6.
Burley DV. 1994. Settlement Pattern and Tongan Prehistory Reconsiderations From Ha'apai. The Journal of the Polynesian Society 103(4):379-411.

Burr DB, Schaffler MB, and Frederickson RG. 1988. Composition of the cement line and its possible mechanical role as a local interface in human compact bone. Journal of Biomechanics 21(11):939-945.
Campbell IC. 2001. Island Kingdom: Tonga Ancient and Modern. Christchurch, NZ: Cantebury University Press.
Cardoso HFV. 2007. Accuracy of developing tooth length as an estimate of age in human skeletal remains: The deciduous dentition. Forensic Science International 172(1):17-22.
Carneiro C, Curate F, Borralho P, and Cunha E. 2013. Radiographic fetal osteometry: Approach on age estimation for the portuguese population. Forensic Science International 231(1-3):397.e391-397.e395.
Child A. 1995a. Towards an Understanding of the Microbial Decomposition of Archaeological Bone in the Burial Environment. Journal of Archaeological Science 22(2):165.
Child AM. 1995b. Microbial taphonomy of archaeological bone. Studies in Conservation 40(1):19-30.
Cipollaro M, Di Bernardo G, Galano G, Galderisi U, Guarino F, Angelini F, and Cascino A. 1998. Ancient DNA in Human Bone Remains from Pompeii Archaeological Site. Biochemical and Biophysical Research Communications 247(3):901-904.
Clark G, and Reepmeyer C. 2014. Stone architecture, monumentality and the rise of the early Tongan chiefdom. Antiquity 88(342):1244-1260.
Collins MJ, Nielsen–Marsh CM, Hiller J, Smith CI, Roberts JP, Prigodich RV, Wess TJ, Csapò J, Millard AR, and Turner–Walker G. 2002. The survival of organic matter in bone: a review. Archaeometry 44(3):383-394.
Collins MJ, Riley MS, Child AM, and Turner-Walker G. 1995. A Basic Mathematical Simulation of the Chemical Degradation of Ancient Collagen. Journal of Archaeological Science 22(2):175-183.
Cooper DML, Thomas CDL, and Clement J, G. 2012. Technological Developments in the Analysis of Cortical Bone History: The Third Dimension and Its Potential in Anthropology. In: Crowder C, and Stout SD, editors. Bone Histology: An Anthropological Perspective. Florida, United States of America: CRC Press. p 361-376.
Cunningham C, Scheuer L, and Black S. 2016. Developmental Juvenile Osteology. Saint Louis: Elsevier Science.
Dal Sasso G, Maritan L, Usai D, Angelini I, and Artioli G. 2014. Bone diagenesis at the micro-scale: Bone alteration patterns during multiple burial phases at Al Khiday (Khartoum, Sudan) between the Early Holocene and the II century AD. Palaeogeography, Palaeoclimatology, Palaeoecology 416:30-42.
Damann FE, and Carter DO. 2013. Human Decomposition Ecology and Postmortem Microbiology. In: Pokines JT, and Symes SA, editors. Manual of Forensic Taphonomy. London: CRC Press. p 37-49.
Davidson JM. 1969. Archaeological excavations in two burial mounts at ‘Atele, Tongatapu. Records of the Auckland Institute and Museum 6(4/6):251-286.
Davis PG. 1997. The bioerosion of bird bones. International Journal of Osteoarchaeology 7(4):388-401.

De Boer HH, Aarents MJ, and Maat GJR. 2013. Manual for the Preparation and Staining of Embedded Natural Dry Bone Tissue Sections for Microscopy. International Journal of Osteoarchaeology 23(1):83-93.
Demirjian A. 1978. Dentition. In: Falkner F, and Tanner J, editors. Human Growth. New York: Plenum Press. p 413-444.
Evans JA. 1987. Comparative dental conditions in several prehistoric Oceanic groups : a thesis submitted for the degree of Master of Arts in Anthropology. Dunedin, New Zealand: University of Otago.
Farah RA, Painuthara L, Broadbent JM, Buckley HR, and Halcrow SE. 2016. Physiological insult or the burial environment: Differentiating developmental defects from post-mortem stained enamel in deciduous dentition from the Chiefdom Period of Tonga, Polynesia. Matters Select 2(6):1-4.
Fazekas IG, and Kósa F. 1978. Forensic Fetal Osteology. Budapest, Hungary: Akadémiai Kiadó.
Ferdon EN. 1987. Early Tonga: as the explorers saw it 1616-1810. Tucson: University of Arizona Press.
Ferretti P, Pasolli E, Tett A, Asnicar F, Gorfer V, Fedi S, Armanini F, Truong DT, Manara S, Zolfo M et al. . 2018. Mother-to-Infant Microbial Transmission from Different Body Sites Shapes the Developing Infant Gut Microbiome. Cell Host & Microbe 24(1):133-145.e135.
Fogel ML, Tuross N, Johnson BJ, and Miller GH. 1997. Biogeochemical record of ancient humans. Organic Geochemistry 27(5):275-287.
Fogel ML, Tuross N, and Owsley DW. 1989. Nitrogen isotope tracers of human lactation in modern and archaeological populations. Carnegie Institution of Washington Yearbook 88:111-117.
Foster AV. 2012. Gendered Divisions of Labour in Southeast Asian and Pacific Island Prehistory a thesis submitted for the degree of Doctor of Philosophy. Dunedin, New Zealand.: University of Otago.
Fratzl-Zelman N, and Varga F. 2017. Osteoblasts and Osteocytes: Essentials and Methods. In: Pietschmann P, editor. Principles of Bone and Joint Research. Cham: Springer International Publishing. p 17-31.
Fry EI. 1976. Dental Development in Cook Island Children. In: Giles E, and Friedlaender JS, editors. The Measures of Man: Methodologies in Biological Anthropology. Cambridge, MA: Peabody Museum Press. p 164-180.
Fuller BT, Fuller JL, Harris DA, and Hedges REM. 2006a. Detection of breastfeeding and weaning in modern human infants with carbon and nitrogen stable isotope ratios. American Journal of Physical Anthropology 129(2):279-293.
Fuller BT, Fuller JL, Sage NE, Harris DA, O'Connell TC, and Hedges REM. 2004. Nitrogen balance and delta15N: why you're not what you eat during pregnancy. Rapid communications in mass spectrometry : RCM 18(23):2889-2896.
Fuller BT, Molleson TI, Harris DA, Gilmour LT, and Hedges REM. 2006b. Isotopic Evidence for Breastfeeding and Possible Adult Dietary Differences from Late/Sub-Roman Britain. American Journal of Physical Anthropology 129(1):45-54.
Garland AN. 1987. A histological study of archaeological bone decomposition. In: Garland AN, and Janaway RC, editors. Death, decay and reconstruction. Manchester, UK: Manchester University Press. p 109-126.
Gartner LP. 2017. Textbook of histology. Philadelphia, Pennsylvania: Elsevier.

Gill-King H. 1997. Chemical and ultrastructural aspects of decomposition. In: Haglund WD, and Sorg MH, editors. Forensic taphonomy: the postmortem fate of human remains. Florida, the United states of America: CRC Press. p 93-108.
Goodman AH, and Armelagos GJ. 1989. Infant and childhood morbidity and mortality risks in archaeological populations. World Archaeology 21(2):225-243.
Gowland R. 2020. Ruptured: Reproductive Loss, Bodily Boundaries, Time and the Life Course in Archaeology. In: Gowland R, and Halcrow SE, editors. The Mother-Infant Nexus in Anthropology: Small Beginning Significant Outcomes. Cham, Switzerland: Springer Nature. p 257-274.
Gowland RL. 2015. Entangled lives: Implications of the developmental origins of health and disease hypothesis for bioarchaeology and the life course. American Journal of Physical Anthropology 158(4):530-540.
Green RC. 1973. Tonga's Prehistoric Population. Pacific Viewpoint 14(1):61-74.
Groer MW, Luciano AA, Dishaw LJ, Ashmeade TL, Miller E, and Gilbert JA. 2014. Development of the preterm infant gut microbiome: a research priority. Microbiome 2(1):38.
Guarino FM, Angelini F, Vollono C, and Orefice C. 2006. Bone preservation in human remains from the Terme del Sarno at Pompeii using light microscopy and scanning electron microscopy. Journal of Archaeological Science 33(4):513-520.
Gurevitch O, Slavin S, and Feldman AG. 2007. Conversion of red bone marrow into yellow – Cause and mechanisms. Medical Hypotheses 69(3):531-536.
Hackett CJ. 1981. Microscopical Focal Destruction (Tunnels) in Exhumed Human Bones. Medicine, Science and the Law 21(4):243-265.
Halcrow SE, and Tayles N. 2011. The Bioarchaeological Investigation of Children and Childhood. In: Agarwhal SC, and Glencross BA, editors. Social Bioarchaeology. Chichester, West Sussex, UK; Malden, MA Wiley-Blackwell. p 333-360.
Halcrow SE, Tayles N, and Buckley HR. 2007. Age estimation of children from prehistoric Southeast Asia: are the dental formation methods used appropriate? Journal of Archaeological Science 34(7):1158-1168.
Halcrow SE, Tayles N, and Elliott GE. 2017. The bioarchaeology of fetuses. In: Han S, Betsinger TK, and Scott AB, editors. The anthropology of the fetus: Biology, culture, and society. New York: Berghahn Books. p 83-111.
Halcrow SE, Tayles NG, and Livingstone V. 2008. Infant Death in Late Prehistoric Southeast Asia. Asian Perspectives 47(2):371-404.
Hanson DB, and Buikstra JE. 1987. Histomorphological alteration in buried human bone from the lower Illinois Valley: Implications for palaeodietary research. Journal of Archaeological Science 14(5):549-563.
Hedges REM. 2002. Bone diagenesis: an overview of processes. Archaeometry 44(3):319-328.
Hedges REM, and Millard AR. 1995. Bones and Groundwater: Towards the Modelling of Diagenetic Processes. Journal of Archaeological Science 22(2):155-164.
Hedges REM, Millard AR, and Pike AWG. 1995. Measurements and Relationships of Diagenetic Alteration of Bone from Three Archaeological Sites. Journal of Archaeological Science 22(2):201-209.
Henderson J. 1987. Factors determining the state of preservation of human remains. In: Boddington A, Garland AN, and Janaway RC, editors. Death, decay and reconstruction. Mancester, UK: Manchester University Press. p 43-54.

Hillson S. 1996. Dental anthropology. Cambridge, England; New York: Cambridge University Press.
Hollund HI, Jans MME, Collins MJ, Kars H, Joosten I, and Kars SM. 2012. What Happened Here? Bone Histology as a Tool in Decoding the Postmortem Histories of Archaeological Bone from Castricum, The Netherlands. International Journal of Osteoarchaeology 22(5):537-548.
Hoppa R. 2000. Population Variation in Osteological Aging Criteria: An Example From the Public Symphysis. American Journal of Physical Anthropology 111(2):185.
Hulland SA, Lucas JO, Wake MA, and Hesketh KD. 2000. Eruption of the primary dentition in human infants: a prospective descriptive study. Pediatr Dent 22(5):415-421.
Ingram RT, Clarke BL, Fishert LW, and Fitzpatrick LA. 1992. Distribution of noncollagenous matrix proteins (NCPS) in the calcified matrix of normal bone: Evidence of anatomical and functional heterogeneity. Bone 13(5):A15.
Janaway RC. 1987. The preservation of organic materials in association with metal artefacts deposited in inhumation graves. In: Boddington A, and Garland AN, editors. Death, decay and reconstruction. Manchester, UK: Manchester University Press. p 127-148.
Jans MME. 2008. Microbial bioerosion of bone - a review. In: M W, and Tapanila L, editors. Current Developments in Bioerosion. Berlin: Springer. p 397-414.
Jans MME, Kars H, Nielsen–Marsh CM, Smith CI, Nord AG, Arthur P, and Earl N. 2002. In situ preservation of archaeological bone: a histological study within a multidisciplinary approach. Archaeometry 44(3):343-352.
Jans MME, Nielsen-Marsh CM, Smith CI, Collins MJ, and Kars H. 2004. Characterisation of microbial attack on archaeological bone. Journal of Archaeological Science 31(1):87-95.
Jelliffe DB. 1967. Parallel food classifications in developing and industrialized countries. The American journal of clinical nutrition 20(3):279-281.
Jiménez E, Fernández L, Marín M, Martín R, Odriozola J, Nueno-Palop C, Narbad A, Olivares M, Xaus J, and Rodríguez J. 2005. Isolation of Commensal Bacteria from Umbilical Cord Blood of Healthy Neonates Born by Cesarean Section. Current Microbiology 51(4):270-274.
Jiménez E, Marín ML, Martín R, Odriozola JM, Olivares M, Xaus J, Fernández L, and Rodríguez JM. 2008. Is meconium from healthy newborns actually sterile? Research in Microbiology 159(3):187-193.
Katzenberg M. 2008. Stable isotope analysis: A tool for studying past diet, demography, and life history. In: Katzenberg M, and Saunders SR, editors. Biological anthropology of the human skeleton. 2nd ed. Hoboken: Wiley. p 413-441.
Kellerman GD, Waterman NG, and Scharfenberger LF. 1976. Demonstration in Vitro of Postmortem Bacterial Transmigration. American Journal of Clinical Pathology 66(5):911-915.
Kendall C, Eriksen AMH, Kontopoulos I, Collins MJ, and Turner-Walker G. 2018. Diagenesis of archaeological bone and tooth. Palaeogeography, Palaeoclimatology, Palaeoecology 491:21-37.
Kibblewhite M, Tóth G, and Hermann T. 2015. Predicting the preservation of cultural artefacts and buried materials in soil. Science of The Total Environment 529:249-263.

Kinaston RL, Buckley HR, Halcrow SE, Spriggs MJT, Bedford S, Neal K, and Gray A. 2009. Investigating foetal and perinatal mortality in prehistoric skeletal samples: a case study from a 3000-year-old Pacific Island cemetery site. Journal of Archaeological Science 36(12):2780-2787.
Kirch PV. 1990. Monumental Architecture and Power in Polynesian Chiefdoms: A Comparison of Tonga and Hawaii. World Archaeology 22(2):206-222.
Kirch PV. 2017. On the road of the winds : an archaeological history of the Pacific islands before European contact. Oakland, California.
Knodel J, and Kintner H. 1977. The Impact of Breast Feeding Patterns on the Biometric Analysis of Infant Mortality. Demography 14(4):391-409.
Konigsberg LW, and Holman D. 1997. Estimation of age at death from dental emergence and implications for studies of prehistoric somatic growth. In: Larsen CS, editor. Bioarchaeology: Interpreting Behaviour from the Human Skeleton. Cambridge: Cambridge University Press. p 264-289.
Kronfeld R. 1935. Development and calcification of the human deciduous and permanent dentition. International Journal of Orthodontia and Dentistry for Children 21(6):592-593.
Kronfeld R, and Schour I. 1939. Neonatal Dental Hypoplasia. The Journal of the American Dental Association 26(1):18-32.
Lampl M, and Johnston FE. 1996. Problems in the aging of skeletal juveniles: Perspectives from maturation assessments of living children. American Journal of Physical Anthropology 101(3):345-355.
Latham KE, and Madonna ME. 2013. DNA Survivability in Skeletal Remains. In: Pokines J, and Symes SA, editors. Manual of Forensic Taphonomy. London: CRC Press. p 403-425.
Lewis M. 2007. The bioarchaeology of children: perspectives from biological and forensic anthropology. Cambridge.
Lewis ME. 2019. Children in Bioarchaeology: Methods and Interpretations. In: Katzenberg MA, and Grauer AL, editors. Biological Anthropology of the Human Skeleton. Chincester, England: John Wiley & Sons. p 119-144.
Lewis ME, and Gowland R. 2007. Brief and precarious lives: Infant mortality in contrasting sites from medieval and post-medieval England (AD 850–1859. American Journal of Physical Anthropology 134(1):117-129.
Liversidge HM, Dean MC, and Molleson TI. 1993. Increasing human tooth length between birth and 5.4 years. American Journal of Physical Anthropology 90(3):307-313.
Logan WHG, and Kronfeld R. 1933. Development of the Human Jaws and Surrounding Structures from Birth to the Age of Fifteen Years. The Journal of the American Dental Association 20(3):379-428.
Lukacs JR, and Largaespada LL. 2006. Explaining sex differences in dental caries prevalence: Saliva, hormones, and “life-history” etiologies. American Journal of Human Biology 18(4):540-555.
Mackie RI, Sghir A, and Gaskins HR. 1999. Developmental microbial ecology of the neonatal gastrointestinal tract. The American journal of clinical nutrition 69(5):1035S-1045S.
Malm T. 2007. Bendable facts: A note on the division of labour in Tonga. In: Vunisea A, editor. Women in fisheries: information bulletin. Noumea, New Caledonia: Secretariat of the Pacific Community. p 3-9.

Marchiafava V, Bonucci E, and Ascenzi A. 1974. Fungal osteoclasia: a model of dead bone resorption. Calcified Tissue Research 14(1):195-210.
Mariner W, and Martin J. 1981. An account of the natives of the Tonga Islands in the South Pacific Ocean, with an original grammar and vocabulary of their language. Vava'u, Tonga: Vava'u Press.
Matamoros S, Gras-Leguen C, Le Vacon F, Potel G, and de La Cochetiere M-F. 2013. Development of intestinal microbiota in infants and its impact on health. Trends in Microbiology 21(4):167-173.
Mays S. 2010. Archaeology of Human Bones. New York.
McKern WC. 1929. Archaeology of Tonga. Honolulu, Hawaii: Bernice P. Bishop Museum.
Melvin J, Cronholm L, Simson L, and Isaacs A. 1984. Bacterial Transmigration as an Indicator of Time of Death. Journal of Forensic Sciences 29(2):412-417.
Merchant VL, and Ubelaker DH. 1977. Skeletal growth of the protohistoric Arikara. American Journal of Physical Anthropology 46(1):61-72.
Millard A. 2001. The Deterioration of Bone. In: Brothwell DR, and Pollard AM, editors. Handbook of Archaeological Sciences. New York: J. Wiley. p 637-647.
Mills A. 2009. 'Akau Tau: Contextualising Tongan War-Clubs. Journal of the Polynesian Society 118(1):7-45.
Miszkiewicz JJ, and Mahoney P. 2017. Human Bone and Dental Histology in an Archaeological Context. In: Errickson D, and Thompson T, editors. Human Remains: Another Dimension: Academic Press. p 29-43.
Moorrees CF, Fanning EA, and Hunt EE. 1963a. Formation and Resorption of Three Deciduous Teeth in Children. American journal of physical anthropology 21(2):205.
Moorrees CFA, Fanning EA, and Hunt EE. 1963b. Age Variation of Formation Stages for Ten Permanent Teeth. Journal of Dental Research 42(6):1490-1502.
Morton LH. 1996. Becoming Tongan: an ethnography of childhood. Honolulu: University of Hawai'i Press.
Mueller NT, Bakacs E, Combellick J, Grigoryan Z, and Dominguez-Bello MG. 2015. The infant microbiome development: mom matters. Trends in Molecular Medicine 21(2):109-117.
Nanci A. 1999. Content and Distribution of Noncollagenous Matrix Proteins in Bone and Cementum: Relationship to Speed of Formation and Collagen Packing Density. Journal of Structural Biology 126(3):256-269.
Nicholson RA. 1996. Bone Degradation, Burial Medium and Species Representation: Debunking the Myths, an Experiment-based Approach. Journal of Archaeological Science 23(4):513-533.
Nielsen-Marsh CM, and Hedges REM. 2000. Patterns of Diagenesis in Bone I: The Effects of Site Environments. Journal of Archaeological Science 27(12):1139-1150.
Nielsen-Marsh CM, Smith CI, Jans MME, Nord A, Kars H, and Collins MJ. 2007. Bone diagenesis in the European Holocene II: taphonomic and environmental considerations. Journal of Archaeological Science 34(9):1523-1531.
Nitsch EK, Humphrey LT, and Hedges REM. 2011. Using stable isotope analysis to examine the effect of economic change on breastfeeding practices in Spitalfields, London, UK. American Journal of Physical Anthropology 146(4):619-628.

OMNI. n.d. Confocal microscopy equipment and specifications. Otago Micro and Nanoscale Imaging.
Owsley DW, and Jantz RL. 1985. Long bone lengths and gestational age distributions of post-contact period Arikara Indian perinatal infant skeletons. American Journal of Physical Anthropology 68(3):321-328.
Page D. 2008. Anatomy of a medical school: a history of medicine at the University of Otago, 1875-2000. Dunedin, N.Z.: Lancaster.
Penders J, Thijs C, Vink C, Stelma FF, Snijders B, Kummeling I, van den Brandt PA, and Stobberingh EE. 2006. Factors Influencing the Composition of the Intestinal Microbiota in Early Infancy. Pediatrics 118(2):511.
Piepenbrink H. 1986. Two examples of biogenous dead bone decomposition and their consequences for taphonomic interpretation. Journal of Archaeological Science 13(5):417-430.
Pietrusewsky M. 1969. An osteological study of cranial and infracranial remains from Tonga. Records of the Auckland Institute and Museum 6(4/6):287-402.
Pinhasi R, and Bourbou C. 2007. How Representative Are Human Skeletal Assemblages for Population Analysis? In: Pinhasi R, and Mays S, editors. Advances in Human Palaeopathology. Chichester, England; Hoboken, NJ: John Wiley & Sons. p 31-44.
Pokines J. 2013. Introduction: Collection of Macroscopic Osseous Taphonomic Data and the Regonition of Taphonomic Suites of Characteristics. In: Pokines J, and Symes SA, editors. Manual of Forensic Taphonomy. London: CRC Press. p 1-14.
Pokines JT, and Baker JE. 2013. Effects of Burial Environment on Osseous Remains. In: Pokines JT, and Symes SA, editors. Manual of Forensic Taphonomy. London: CRC Press. p 73-114.
Poulsen J. 1974. Archaeology and Ethnic Problems. Mankind 9(4):260-267.
Quinn CJ. 1990. Stable isotopes and diet: indications of the marine and terrestrial component in the diets of prehistoric populations from New Zealand and the Pacific: a thesis submitted for the degree of Master of Arts in anthropology. Dunedin, New Zealand: University of Otago.
Riedel CU, Schwiertz A, and Egert M. 2014. The Stomach and Small and Large Intestinal Microbiomes. In: Marchesi JR, editor. The Human Microbiota and Microbiome. Oxfordshire, UK; Boston, MA: CAB International. p 1-19.
Robb KF, Buckley HR, Spriggs M, and Bedford S. 2012. Cortical index of three prehistoric human Pacific Island samples. International Journal of Osteoarchaeology 22(3):284-293.
Roberts SJ, Smith C, Millard A, and Collins M. 2002. The taphonomy of cooked bone: Characterizing boiling and its physico-chemical effects. Archaeometry 44:485-494.
Roschger P, Blouin S, Paschalis E, Gamsjaeger S, Klaushofer K, and Misof B. 2017. Bone Material Quality. In: Pietschmann P, editor. Principles of Bone and Joint Research. Cham: Springer International Publishing. p 1-15.
Rucci N, and Teti A. 2017. Osteoclasts: Essentials and Methods. In: Pietschmann P, editor. Principles of Bone and Joint Research. Cham: Springer International Publishing. p 33-53.
Rühli FJ, Kuhn G, Evison R, Müller R, and Schultz M. 2007. Diagnostic value of micro-CT in comparison with histology in the qualitative assessment of

historical human skull bone pathologies. American Journal of Physical Anthropology 133(4):1099-1111.
Saunders SR. 1992. Subadult skeletons and growth related studies. In: Saunders SR, and Katzenberg M, editors. Skeletal Biology of Past Peoples: Research Methods. New York: Wiley-Liss. p 1-20.
Saunders SR. 2008. Juvenile Skeletons and Growth-Related Studies. In: Katzenberg MA, and Saunders SR, editors. Biological Anthropology of the Human Skeleton. Chincester, England: John Wiley & Sons. p 117-147.
Scheuer JL, Musgrave JH, and Evans SP. 1980. The estimation of late fetal and perinatal age from limb bone length by linear and logarithmic regression. Annals of Human Biology 7(3):257-265.
Scheuer L, and Black S. 2000. Development and Ageing of the Juvenile Skeleton. In: Cox M, and Mays S, editors. Human Osteology in Archaeology and Forensic Science. London, England: Greenwich Medical Media Ltd. p 9-22.
Schotsmans EMJ, Denton J, Fletcher JN, Janaway RC, and Wilson AS. 2014. Short-term effects of hydrated lime and quicklime on the decay of human remains using pig cadavers as human body analogues: Laboratory experiments. Forensic Science International 238:142.e141-142.e110.
Schotsmans EMJ, Márques-Grant N, and Forbes SL. 2017. Introduction. In: Schotsmans EMJ, Márques-Grant, and Forbes SL, editors. Taphonomy of Human Remains: Forensic Analysis of the Dead and the Depositional Environment. Chichester, England: John Wiley & Sons Ltd. p 1-7.
Schour I, and Massler M. 1941. The Development of the Human Dentition. Journal of the American Dental Association 28:1153-1160.
Schultz M. 2001. Paleohistopathology of bone: A new approach to the study of ancient diseases. American Journal of Physical Anthropology 116(S33):106-147.
Scott RM. 2008. Skeletal trauma in prehistoric Oceania: a biocultural study using samples from Papua New Guinea, Solomon Islands, Vanuatu, Tonga, and New Zealand: a thesis submitted for the degree of Master of Science. Dunedin, New Zealand: University of Otago
Scott RM, and Buckley HR. 2014. Exploring Prehistoric Violence in Tonga. Current Anthropology 55(3):335-347.
Scott S, and Duncan CJ. 1999. Malnutrition, Pregnancy, and Infant Mortality: A Biometric Model. Journal of Interdisciplinary History 30(1):37-60.
Siebke I, Moghaddam N, Cunningham CA, Witzel C, and Lösch S. 2019. Those who died very young-Inferences from δ15 N and δ13 C in bone collagen and the absence of a neonatal line in enamel related to the possible onset of breastfeeding. American journal of physical anthropology 169(4):664-677.
Smith CA, Lowe BJ, Blair K, Carr D, and McNaughton A. 2013. Identification of historical plant material using micro-computed tomography. Studies in Conservation 58(3):256-268.
Smith CI, Nielsen-Marsh CM, Jans MME, and Collins MJ. 2007. Bone diagenesis in the European Holocene I: patterns and mechanisms. Journal of Archaeological Science 34(9):1485-1493.
Smith CI, Nielsen–Marsh CM, Jans MME, Arthur P, Nord AG, and Collins MJ. 2002. The strange case of Apigliano: early ‘fossilization’ of medieval bone in southern Italy. Archaeometry 44(3):405-415.

Southorn M. 2019. Forgotten Children: The Infant and Foetal Skeletal Remains of the W.D. Trotter Anatomy Museum: A thesis submitted for the degree of Bachelor of Science with Honours. Dunedin, New Zealand: University of Otago.
Stantis C. 2015. Diet and migration in prehistoric remote Oceania : a thesis submitted for the degree of Doctor of Philosophy at the University of Otago, Dunedin, New Zealand.
Stantis C, Kinaston R, Richards M, Davidson J, and Buckley H. 2015. Assessing Human Diet and Movement in the Tongan Maritime Chiefdom Using Isotopic Analyses. PLoS One 10(3):1-27.
Steiniche T, and Hauge Em. 2004. Normal Structure and Function of Bone. In: Claassen H, editor. Handbook of Histology Methods for Bone and Cartilage. Germany, Jena: Urban & Fischer Verlag. p 59-72.
Stevens A, and Lowe JS. 2005. Human histology. Philadelphia; London: Elsevier Mosby.
Stodder ALW. 2019. Taphonomy and the nature of archaeological assemblages. In: Katzenberg M, and Grauer AL, editors. Biological Anthropology of the Human Skeleton. Hoboken, New Jersey: John Wiley & Sons, Inc. p 73-115.
Storey AA. 2008. Tools of the Ancestors? Evidence for Culturally Modified Human Bone from Tongan Skeletal Assemblages. In: Addison DJ, and Sand C, editors. Recent Advances in the Archaeology of the Fiji/West-Polynesia Region. 21 ed. Dunedin: Department of Anthropology, Gender and Sociology, University of Otago.
Suren P. 2009. Essays on the history of the discovery and exploration of Tonga by the Europeans. Nukuʻalofa, Tonga: Friendly Islands Bookshop.
Symes SA, L'abbé EN, Pokines JT, Yuzwa T, Messer D, Stromquist A, and Keough N. 2013. Thermal Alteration to Bone. In: Pokines JT, and Symes SA, editors. Manual of Forensic Taphonomy. London: CRC Press. p 367-402.
Te Moananui R, Kieser JA, Herbison P, and Liversidge HM. 2008. Advanced dental maturation in New Zealand Maori and Pacific Island children. American Journal of Human Biology 20(1):43-50.
Tocheri MW, Dupras TL, Sheldrick P, and Molto JE. 2005. Roman Period fetal skeletons from the East Cemetery (Kellis 2) of Kellis, Egypt. International Journal of Osteoarchaeology 15(5):326-341.
Trueman CN, and Martill DM. 2002. The long–term survival of bone: the role of bioerosion. Archaeometry 44(3):371-382.
Trujillo-Mederos A, Alemán I, Botella M, and Bosch P. 2012. Changes in human bones boiled in seawater. Journal of Archaeological Science 39(4):1072-1079.
Turner-Walker G. 2008. The Chemical and Microbial Degradation of Bones and Teeth. Advances in Human Palaeopathology. p 3-29.
Turner-Walker G, and Jans M. 2008. Reconstructing taphonomic histories using histological analysis. Palaeogeography, Palaeoclimatology, Palaeoecology 266(3):227-235.
Ubelaker DH. 1989. Human skeletal remains : excavation, analysis, interpretation. Washington: Taraxacum.
Van Der Grijp P. 1993. The making of a modern chiefdom state: the case of Tonga. Bijdragen tot de Taal-, Land- en Volkenkunde 149(4):661-672.
Waldron T. 1987. The relative survival of the human skeleton: implications for palaeopathology. In: Boddington A, Garland AN, and Janaway RC, editors.

Death, decay and reconstruction. Mancester, UK: Manchester University Press. p 55-64.
Waldron T. 1994. Counting the dead : the epidemiology of skeletal populations. Chichester, New York: John Wiley & Sons.
Walsh WR, Walton M, Bruce W, Yu Y, Gillies RM, and Svehla M. 2004. Cell Structure and Biology of Bone and Cartilage. In: Claassen H, editor. Handbook of Histology Methods for Bone and Cartilage. Germany, Jena: Urban & Fischer Verlag. p 35-58.
Weather-and-Climate. 2019. Climate and Average Weather in Tonga.
Weiss-Krejci E. 2001. Restless corpses: 'secondary burial' in the Babenberg and Habsburg dynasties. Antiquity 75(290):769-780.
White EM, and Hannus LA. 1983. Chemical Weathering of Bone in Archaeological Soils. American Antiquity 48(2):316-322.
White L, and Booth TJ. 2014. The origin of bacteria responsible for bioerosion to the internal bone microstructure: Results from experimentally-deposited pig carcasses. Forensic Science International 239:92-102.
White TD, Black MT, and Folkens PA. 2012. Human osteology. Amsterdam; Boston: Elsevier Academic Press.
White TD, and Folkens PA. 2005. The Human Bone Manual: Elsevier Academic Press.
Whitford D. 2005. Proteins Structure and Function. 1st ed. ed. Hoboken: Wiley.
Willis A, and Oxenham MF. 2013. The neolithic demographic transition and oral health: The Southeast Asian experience. American Journal of Physical Anthropology 152(2):197-208.
Yoshino M, Kimijima T, Miyasaka S, Sato H, and Seta S. 1991. Microscopical study on estimation of time since death in skeletal remains. Forensic Science International 49(2):143-158.