The Differential Degradation of Immature and Mature Bone in Diverse Environments: A Controlled Experiment Using Pig (*Sus scrofa*) Skeletal Remains

by

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Abstract

Several studies suggest that juvenile skeletal remains are significantly underrepresented in both forensic and archaeological excavations. In archaeological contexts, the disparities between historical burial records and the relative absence of juveniles in cemetery excavations have been a cause for much speculation. The most popular explanation for this paucity in the osteological record is a comparatively rapid breakdown of juvenile bones, due to their smaller size, incomplete mineralization, higher organic and water content, and higher porosity than their adult counterparts. If this holds true, it presents a challenge for accurately identifying skeletonized juveniles in forensic cases. While the idea is widely accepted, few experiments have provided evidence to support it.

This study uses infant and sexually mature porcine models to explore the role of bone maturity with regards to: *1) overall susceptibility of the skeleton to biological, physical, and compositional degradation, and 2) the interaction of bone material with different burial environments*. The ulnae of immature (2-8 weeks) and mature (6 months) pigs (*Sus scrofa)* were mechanically defleshed and used as a proxy for human bone of distinct infant and sexually mature groups. Samples (n=200) from both maturity groups were left to degrade in a climate-controlled greenhouse, either buried or on the soil surface. These two varying depositional conditions provide the degradation factors from two different environments. Every month, four bones from each maturity group and environment were collected. Weight loss on ignition analysis was performed on each sample to determine the relative water, collagen, and mineral composition of the bones, and bone weathering analysis was performed to quantify the physical changes of the bone surface.

The results of this study indicate that, in the early postmortem interval, immature and mature bone material are differentially affected by their postmortem depositional environment. In both the subaerial and buried environments, the immature bone was found to be more susceptible to compositional degradation, while the mature bone was more heavily affected by physical weathering. It is not known how these initial differences in bone breakdown translate into the long-term survival of immature bone material, however, this study suggests that any interpretations of weathered immature bone, that are based on weathering rates determined by mature bone, should be done so with caution.

Keywords: Juvenile Osteology; Forensic Science; Taphonomy; Human Osteology; Bone Weathering

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Table of Contents

List of Tables

List of Figures

Chapter 1.

Introduction

Several studies suggest that juvenile remains are significantly underrepresented in both archaeological excavations and forensic investigations (Bello and Andrews 2006, Buckberry 2000, Djuric et al. 2011, Guy 1997, Lewis 2007, Manifold 2010, 2012, 2013, Mays 2010, Walker et al, 1988). In archaeological contexts, the disparities between historical burial records and the relative absence of juveniles in cemetery excavations have been a cause for much speculation (Manifold 2010). The most accepted explanation for this paucity in the archaeological record – in addition to differential burial practices - is a comparatively rapid breakdown of juvenile bones, due to their smaller size, incomplete mineralization, higher organic and water content, and higher porosity when compared to their adult counterparts (Bello and Andrews 2006, Buckberry 2000, Djuric et al. 2011, Gordon and Buikstra 1981, Guy 1997, Lewis 2007, Manifold 2010, 2012, 2013, Mays 2010, Walker et al. 1988). This same reason is used to explain the lack or poor preservation of juvenile remains in forensic investigations (Lewis 2007, Manifold 2012). If this holds true, it presents a challenge for accurately identifying skeletonized juveniles in forensic cases (Donaldson and Lamont 2012, Ferreira and Cunha 2013), and poses a problem for paleodemographic and paleopathological studies (Bello and Andrews 2006, Buckberry 2000, Djuric et al. 2011, Guy 1997, Lewis 2007, Manifold 2010, 2012, 2013, Mays 2010, Walker et al. 1988, Wood et al. 1992). While the idea of differing breakdown rates is widely accepted, few experiments have provided evidence to support it (Djuric et al. 2011, Manifold 2010, Walker et al. 1988). Thus, further research into the matter is needed in both the fields of archaeology and forensics.

1.1. The Archaeological Context

Juvenile skeletons are often recovered in extremely low numbers from cemetery excavations (Bello and Andrews 2006, Buckberry 2000, Djuric et al. 2011, Guy 1997, Lewis 2007, Manifold 2010, 2012, 2013, Mays 2010, Walker et al. 1988). Based on the idea that archaeological societies should be comparable to pre-industrialized ones, it is expected that at least 30% of cemetery remains should be children, however, this is

rarely the case (Akazawa et al. 1995). This confounding paucity in the archaeological record causes problems for paleodemographic studies, which require that a skeletal sample be reflective of the living population of study (Angel 1969, Bello and Andrews 2006, Djuric et al. 2011, Lovejoy 1971, Manifold 2010, Roksandic and Armstrong 2011, Walker et al. 1988), as well as paleopathological studies, which use a number of stress indicators that affect the developing skeleton to infer population health (Krenz-Niedbata 2017). While researchers recognize and attempt to compensate for this deviation from expected population structures, no single explanation can account for its widespread prevalence (Bello and Andrews 2006, Guy 1997, Lewis 2007).

It is generally accepted that taphonomic processes act most heavily upon juvenile remains, however, the magnitude to which this may occur is still not well understood (Djuric et al. 2011, Manifold 2010, Walker et al. 1988). While many archaeologists favor this idea, regardless of the lack of supporting evidence, variable preservation of archaeological juvenile skeletal material suggests that there are other factors at play (Bello and Andrews 2006, Buckberry 2000, Djuric et al 2011, Gordon and Buikstra 1981, Guy 1997, Lewis 2007, Manifold 2010, 2012, 2013, Mays 2010, Walker et al. 1988). The preserved remains of a 100,000 year old Neanderthal child (Akazawa et al. 1995), and the excellent preservation of juveniles within a 19th century Californian cemetery (Buckberry 2000) are just a few of the cases that have caused archaeologists to reconsider the situation. The alternative ideas that have been put forward include the differential treatment of children in past cultures, and the modern archaeological techniques that are not often tailored towards retrieving juvenile remains (Bello and Andrews 2006, Buckberry 2000, Guy 1997, Lewis 2007, Mays 2010, Manifold 2010, 2012, 2013).

Differential treatment of juvenile remains dates back to the Upper Paleolithic, and has its roots in social organization, folklore, and religion (Buckberry 2000, Lewis 2007). These differing funerary practices dictated the burial location and depth of juvenile remains, thus influencing the preservation of their skeletal material (Buckberry 2000, Lewis 2007, Mays 2010). In German folklore, for instance, it was believed that evil forces would swap human children for their wicked offspring (Gardela and Duma 2013). To rid the community of the devil, children with visible deformities or disabilities would be murdered and given a clandestine burial outside the community funeral grounds (Gardela 2013). In 17th century English Catholic societies, neonates could not be buried

on consecrated cemetery grounds unless a baptism and funeral were paid for, resulting in their burial along the outside of cemetery walls (Guy 1997, Lewis 2007). Several archaeological excavations in Greece, England, and across the Near East, have documented the burial of children under house floorboards, instead of in the main burial areas (Fernandez-Crespo 2014, Guy 1997, Lewis 2007). Practices such as these cause children to be buried in less protected shallow graves and in a less uniform distribution across cemeteries than would normally be expected of a population; resulting in cemetery excavations that inadvertently exclude juvenile burials.

The processes involved in archaeological excavation and curation have also changed drastically through space and time, resulting in differing degrees of bias in paleodemographic reports. Prior to the 1990s, children were deemed unimportant in most paleodemographic studies due to their poor representation in the archaeological record (Buckberry 2000, Djuric et al. 2011, Manifold 2010). Reduced reporting and publishing on children in archaeological projects resulted in an apparent lack of that age group in cemetery excavations (Manifold 2010). In Scandinavia, for example, only intact human crania were collected for museum displays, which resulted in the complete exclusion of unfused juvenile cranial bones (Lewis 2007). Since this time, researchers have realized the value in studying children, however, their techniques are not always conducive to a thorough retrieval and understanding (Buckberry 2000). The comprehensiveness of juvenile bone retrieval is often quite poor due to the irregular shape and small size of the bones (Manifold 2010). This problem is exacerbated by the fact that most recovery sheets do not outline the shape and number of juvenile bones as they change throughout the developmental process (Manifold 2010). Other field methods such as excavation location, screening, and rough handling of skeletal remains can also result in a lack of juvenile material being retrieved due to accidental destruction or exclusion (Buckberry 2000, Henderson 1987, Lewis 2007, Manifold 2012, Mays 2010, Saunders 2008).

It is evident that the actions of humans have a great impact on the preservation of bone, in both the pre-burial and post-burial contexts (Bello and Andrews 2006). The lack of juvenile skeletons in the archaeological record can be easily attributed to anthropogenic factors, which makes the assertion of their preferential taphonomic destruction in need of justification. This thesis will address the matter by testing the assumption that immature bone degrades more quickly than mature bone.

1.2. The Forensic Context

The determination of postmortem interval, or time-since-death, of human remains is a critical aspect of solving homicide cases and identifying missing persons (Donaldson and Lamont 2012, Ferreira and Cunha 2013, Kumar et al. 2015, Maile et al. 2017, Wilson and Christensen 2017). Such determinations provide a timeline, which aids in ruling out suspects and helps narrow down the possible identification pool of victims (Donaldson and Lamont 2012, Ferreira and Cunha 2013, Wilson and Christensen 2017). The search for an accurate way to determine time-since-death began as early as 2000 years ago, with the Egyptians performing autopsies and dissections (Donaldson and Lamont 2012). Unfortunately, the vast numbers of factors that act upon human remains have resulted in inaccurate, non-specific, and subjective methods of analyzing the postmortem interval (Bilheux et al. 2015, Boaks et al. 2014, Donaldson and Lamont 2012, Vass 2011, Wilson and Christensen 2017). Furthermore, the accuracy of estimating this interval decreases with decomposition time, making skeletonized remains nearly impossible to use with any precision (Boaks et al. 2014, Goff 2009).

Estimating the postmortem interval in children, specifically, has been poorly researched in comparison to adults. This is simply due to the lack of access to juvenile skeletal materials and the forensic cases involving them (Lewis 2007). While decomposition of adult remains is well documented, their established rate of decay may not be applicable to children (Neideregger et al. 2017). Inaccurate estimations of time-since-death can lead to the misidentification of a victim and the incorrect prosecution of a criminal. To fill this void in forensic research, this project will provide the relative rates of compositional and physical degradation of juvenile bone, when directly compared to those of mature bone.

1.3 Research Objective

This study systematically explores the effects of the length of the postmortem interval on the compositional and physical weathering of immature and mature bone in two different depositional environments. The role of bone composition and maturity is explored, with regards to: *1) overall susceptibility to degradation, and 2) interaction of*

degradation with different depositional environments. If immature bone is more susceptible to degradation, measures of compositional and physical breakdown will be markedly greater relative to mature bone under identical conditions. Given the hypothesized differences in degradation between the two types of bone, composition and maturity will also likely mediate the magnitude to which environmental factors degrade bone samples within each of the two different depositional environments.

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Chapter 2.

Differential Weathering of Immature and Mature Bone in a Subaerial Environment

This chapter will be submitted for publication in the Journal of Forensic Sciences.

2.1. Abstract

Time-since-death of skeletonized remains is approximated using known trends in bone breakdown that were developed from adult remains. Approximations of time-since-death based on bone breakdown from juvenile remains are not well known. This study uses a porcine model to explore the role of bone maturity with regards to the overall susceptibility of bone to physical and compositional degradation in a subaerial environment. Samples (n=104) were mechanically defleshed and left to degrade in a climate-controlled environment, placed upon the soil surface. Every month, for the span of 12 months, 4 immature and mature bone samples were collected. Weight loss on ignition analysis was performed to determine the compositional changes of the bones, and bone weathering analysis determined their physical breakdown. Results indicate that in the early postmortem interval of subaerial remains, the compositional and physical degradation rates of immature and mature bone material are significantly different. Immature bone were found to be more susceptible to compositional changes and less affected by surface weathering. This suggests that the existing forensic methods of postmortem interval estimation of skeletonized remains may not be reliable for juveniles.

2.2. Keywords (6):

Forensic Science, Human Osteology, Postmortem Interval, Porcine Bone, Bone Taphonomy, Juvenile Osteology.

2.3. Introduction

The determination of postmortem interval, or time-since-death, is a critical aspect of solving homicide cases and identifying human remains (Donaldson and Lamont 2014, Ferreira and Cunha 2013, Kumar et al. 2015, Maile et al. 2017, Wilson and Christensen 2017). It provides a timeline for ruling out suspects and helps to narrow down the possible identification pool of victims (Donaldson and Lamont 2014, Ferreira and Cunha 2013, Kumar et al. 2015, Wilson and Christensen 2017). The search for an accurate way to determine time-since-death began as early as 2000 years ago, with the Egyptians performing autopsies and dissections (Ferreira and Cunha 2013). Unfortunately, the incredible number of factors that act upon human remains have caused the results of many previous studies to be inaccurate, wide-ranging, and subjective (Bilheux et al. 2015, Boaks et al. 2014, Ferreira and Cunha 2013, Vass 2011, Wilson and Christensen 2017).

Taphonomy, a term coined by Efremov in the 1940s, refers to the process through which animal remains transition from the biosphere to the lithosphere (Bello and Andrews 2006, Manifold 2012). This process has been extensively researched, providing a predictable sequence in which decomposition processes take place (Goff 2009, Junod and Pokines 2012). In the earliest stages of decomposition, postmortem interval can be estimated using bodily cooling rates, livor mortis, which is staining caused by blood pooling, or rigor mortis, the stiffening of muscles due to a lack of ATP production (Donaldson and Lamont 2014, Goff 2009). Shortly after, autolysis, or self-digestion from internal enzymes and bacteria, manifests in the form of bloating, skin slippage, and soft tissue destruction (Junod and Pokines 2012, Vass 2001). Insect and scavenger activity also aid in the active destruction of the remains. The colonization and lifecycle of insects in a human body is predictable, allowing for entomological estimates of postmortem interval (Goff 2009, Junod and Pokines 2012). Scavenger activity results in disarticulation and cortical damage, which also happens in a predictable manner, beginning with the extremities (Ubelaker 1997). The final, broadest stage of decomposition is skeletonization, which occurs when all soft tissue has been removed from the bones (Goff 2009, Junod and Pokines 2012, Ubelaker 1997); this occurs earlier in subaerial remains than those that are protected (Behrensmeyer 1978, Goff 2009, Ubelaker 1997). Following skeletonization, weathering processes begin to modify the

structural integrity of the bone (Behrensmeyer 1978, Junod and Pokines 2012, Ubelaker 1997).

Weathering is the first stage in which bone starts to compositionally and physically degrade due to exposure to its burial or depositional environment. In 1978, Behrensmeyer provided a formal definition of bone weathering, stating that it is the process of separating and destroying the microscopic structure of the organic and inorganic components of bone. This research went on to provide the first index for observationally quantifying the effects of the deposition environment on bone material (Behrensmeyer 1978, Lyman and Fox 1989, Ubelaker 1997). This study, however, produced stages that not only introduce discontinuity into a continuous process (Lyman and Fox 1989) but also are specific to large mammals and provide wide non-specific time spans (Haglund and Sorg 1997, Madgewick and Mulville 2012, Tappen 1994). In 1990, Andrews performed the same type of observational study, this time using small rodents, and produced weathering indices that were also overlapping, imprecise, and specific to the animals of study.

Estimating the postmortem interval in children, specifically, has not been well researched in comparison to adults. This is simply due to the lack of access to these materials for experimental studies and the rarity of forensic cases involving juveniles (Lewis 2007). While the decomposition of mature skeletal remains is well documented, their established rates of decay may not be applicable to children due to the nature of their bone chemistry (Neideregger et al. 2017). Immature bones are smaller in size, incompletely mineralized, and have a higher collagen and water content than their adult counterparts (Bello and Andrews 2006, Buckberry 2000, Djuric et al. 2011, Gordon and Buikstra 1981, Guy 1987, Manifold 2010, 2012, 2013, Mays 2010, Walker et al. 1988). These intrinsic features of immature bone material have led researchers to argue that immature skeletal remains degrade faster than those of a mature individual when in comparable depositional conditions (Angel 1969, Bell et al. 1996, Bell and Andrews 2006, Boaks et al. 2014, Djuric et al. 2011, Nielsen-Marsh and Hedges 2000). An experiment by Gonzales et al. (2011) exposed guanco bones of varying maturities to a subaerial environment, and confirmed that age may play a role in determining the rate of bone decay within the first 5 years postmortem. Recently, Cunningham et al. (2011) did an experiment to test the influence of the North Carolina Piedmont environment on immature pig bone weathering within the first year of burial. This research produced

more forensically-applicable bone weathering results than any previous research, given its short postmortem interval. It is worth noting, however, that the aforementioned studies were analyzing the breakdown of animal bone tissue, which is not identical that of humans. Pig bone is accepted as an appropriate proxy for the human bone given its morphological and biological similarities (Cunningham 2011, Turner and Wiltshire 1999), however, the arrangement of their osteons is not identical (Hillier and Bell 2007). The application of such projects, including the current study, to humans must be done with these intrinsic differences in mind. They do, however, inform the forensic estimation of postmortem interval in remains that have reached skeletonization, which is the most difficult to analyze due to its long timespan, the variable changes that occur within it, and the limited standardization of weathering observations (Boaks et al. 2014, Goff 2009). The results of such studies provide a starting point for further research into the effects of intrinsic bone differences on the rate of their physical weathering. Defining these differences is important to the forensic community, as the estimation of time-since-death in immature individuals could be compromised if mature bone weathering rates are used as the reference. Inaccurate estimations of time-since-death can lead to the misidentification of a victim, and the incorrect prosecution of a criminal (Donaldson and Lamont 2014, Ferreira and Cunha 2013).

This project will contribute to forensic knowledge about the physical and compositional degradation rates of subaerial immature and mature bone, within the early postmortem period. The hypothesis being tested is: *if maturity-dependent intrinsic qualities of bone have an effect on the breakdown rate of the material, then the immature and mature bone will exhibit differential reactions in a given depositional environment.* The maturity-dependent intrinsic differences between bone material of varying maturity levels are analyzed as the causative factors for their differential breakdown rates. This is achieved through the compositional and observational analysis of bone weathering in pig bone (n=104), acting as a proxy for the human skeleton, within the first year of degradation. The intrinsic effects are isolated by performing all experiments in a consistent and controlled subaerial environment. The compositional changes are recorded using weight loss on ignition analysis, and the physical changes are observed through bone weathering analysis. The results from this study, while not attempting to create a measure of postmortem interval, will provide comparative

information that can be integrated into the forensic process of analyzing immature remains.

2.4. Materials and Methods

2.4.1. The Experimental Setup

This experiment was designed to directly compare the effects of a consistent subaerial environment on the weathering rate of immature and mature bone material, using pig (*Sus scrofa*) remains. Pigs were chosen as a proxy for humans as they are regularly used in degradation experiments, given the biological and morphological similarity of their skeletons, and their commercial availability (Cunngingham 2011, Turner and Wiltshire 1999). Only the forelimbs of the pigs were used for this project in order to control for intrinsic factors in bone weathering, including size, shape, surface area, and bone density, and due to the ease and affordability of acquiring these bones. The bone samples were obtained from a local meat supplier as fleshed forelimbs from pigs from two distinct maturity groups: immature aged between 2-8 weeks (n=52), and mature aged at 6 months (n=52). The samples were prepared by mechanically defleshing and extracting the radius and ulna from each forelimb, weighing and measuring them, then storing them in labeled bags at 4°C until being brought to the deposition site.

The depositional environment used in this study was confined to a climate-controlled greenhouse, allowing extrinsic factors to be closely monitored across all experimental plots. The experimental plots were created by layering 20cm of lightly packed homogenized organic soil and sand in 60x40x32cm (53L) Rubbermaid® containers with drainage holes, then placing the bone samples on top. Twelve containers were used to provide an experimental plot for each month of the 1-year study period. The plots contained 4 bones from each maturity group and were exposed to the same external factors throughout the duration of the experiment.

Monitoring and control over extrinsic factors were done on a weekly basis, ensuring that the atmospheric temperature and humidity of the greenhouse did not vary immensely with the seasons and that the pH, moisture content, and temperature of the soil did not vary between the different experimental plots. These measurements were

obtained using an ambient measuring device in the greenhouse, and a portable pH, moisture, and temperature probe 10cm deep in the soil. Fluctuations in soil moisture were mitigated with regular watering.

Every 30 days, for the span of one year, the bones from one Rubbermaid® container were collected for analysis. The 4 bones from each maturity group were collected, freed of excess dirt, then transferred into polyethylene bags. Prior to analysis, the section of bone required for weight loss on ignition analysis (see below) was obtained, and then the remainder of the sample was macerated in warm water and tergazyme until all remaining soft tissues were freed from the bone samples. The bagged samples were then photographed and stored at 4°C for the rest of the experimental interval to prevent bacterial growth (Micozzi 1997).

2.4.2. Weight Loss on Ignition

This protocol was designed as an adaptation of previous bone ashing experiments, such as those by Nielsen et al. (1980), Lochmuller et al. (2000), Park et al. (2003), and Pienkowski et al. (2009), in order to detect any compositional breakdown of the bone samples during the one year study period. The methods employed by Lochmuller et al. (2000) were validated using Dual-Energy X-Ray Absorptiometry, while the results of Pienkowski et al. (2009) were validated using Fourier Transform Infrared Spectroscopy. This supports the effectiveness of this protocol to determine the quantities of unbound water, bound water, collagen, and mineral quantities in bone material, while being cost-effective and relatively simple to perform (Fisk et al. 2017).

The ulna of each forelimb in the immature and mature groups was sacrificed to obtain a bone sample of at least $1cm^3$ from the shaft, immediately following collection from their depositional environment. The samples were then heated at room temperature, 65°C, 105°C, and 600°C, and weighed between each increase in temperature. The temperatures were adapted from the aforementioned previous bone ignition studies, and optimized to our sample size and timeframe in order to measure the relative unbound water, bound water, collagen, and mineral contents by weight. The steps are given in Figure 1.

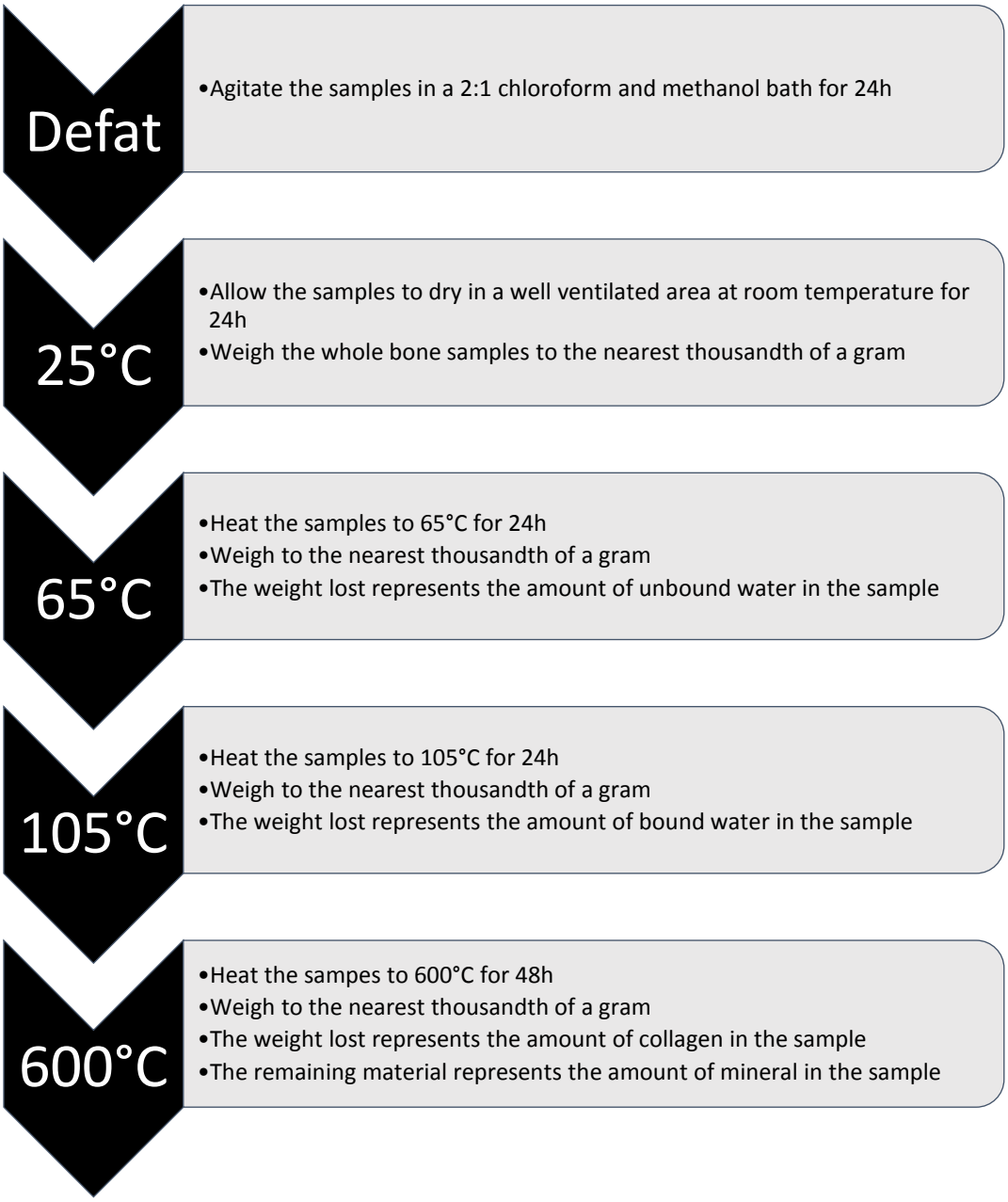


Figure 1: Steps in the weight loss on ignition weight protocol.

Temporal changes in relative proportions of unbound water, bound water, collagen and mineral were examined separately for the immature and mature samples by comparing median values for each sampling time using a Kruskal-Wallis test and post-hoc pairwise comparison with Bonferroni correction. This provided an in-depth analysis of how the bone composition changed during each month, and within each maturity group. The relative proportions of unbound water, bound water, collagen, and

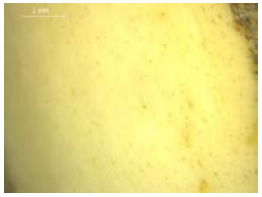
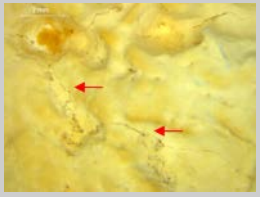
mineral were then compared between the immature and mature bone samples from each separate month by using a Mann-Whitney U test. Additionally, a Kolmogorov Smirnov test was used to determine if the distribution of the weight loss on ignition values through time differed significantly between the two maturity groups. These two tests allowed comparison of the values of each bone component in order to evaluate the intrinsic differences in composition due to maturity, as well as if these differences were maintained through time.

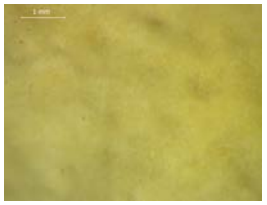
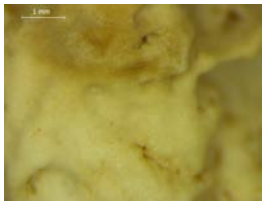
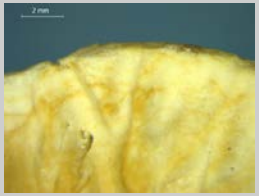
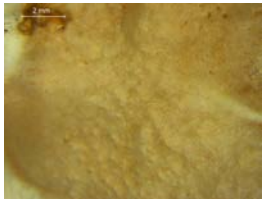
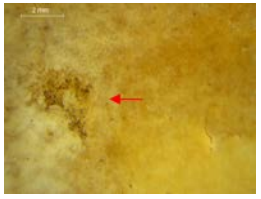
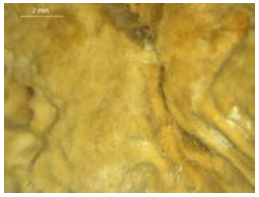
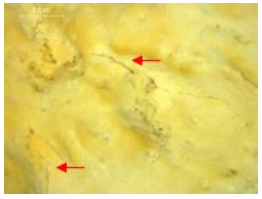
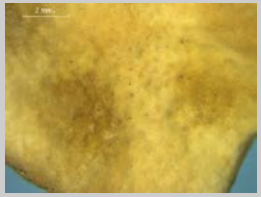
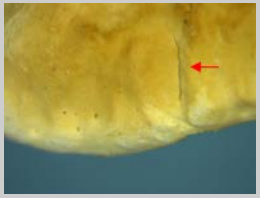
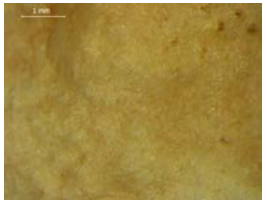
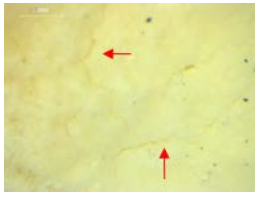
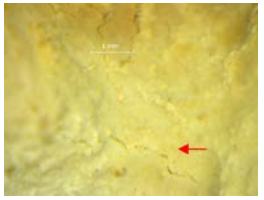
2.4.3. Bone Surface Weathering Analysis

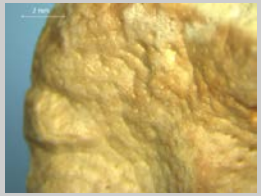
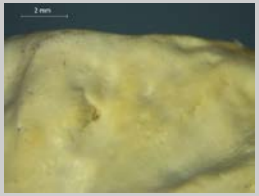
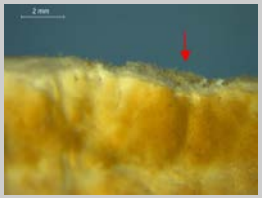
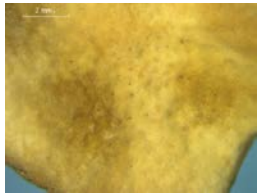
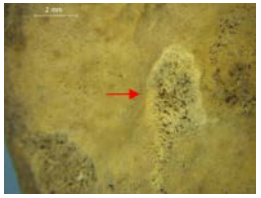
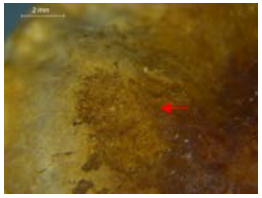
The bone weathering analysis consisted of visually examining the physical changes on the surface of the immature and mature radii samples that occurred over time. After examining all of the bone samples in great detail, a series of surface alterations were identified and categorized to reflect their impact on the superficial bone structure. The variables considered were similar to those in the literature, such as the mosaic cracking observed by Behrensmeyer (1978), and the localized cortical loss referred to as ‘pockmarking’ by Cunngingham and Ross (2011). The selected weathering changes were then included based on their presence in at least one bone sample, and were further split into sub-categories when the affected regions of bone differed through time.

The 9 variables that were examined include cortical peeling, and cracking and loss of the metaphyseal surfaces. Cortical peeling was observed as the lifting and separating of the outermost layer of the bone diaphysis, and was categorized by the region of bone that it affected. Cracking of the metaphyseal surface was denoted by the presence of at least one crack, that was further classified as macroscopic (visible to the naked eye), or microscopic (visible at 10x magnification). Localized loss of the metaphyseal surfaces was considered as the loss of cortical bone to reveal the underlying trabeculae in either the peripheral or central regions of the metaphyseal surfaces. A complete list of the weathering variables used in this project, as well as their illustrations are given in Table 1.

Table 1 **The bone surface weathering variables, their descriptions, and illustrations of their absence and presence. All images depicting the absence of a trait were obtained from fresh bone samples, while those with surface alterations were obtained from bones that decayed in a subaerial environment. Arrows indicate features present.**

Variable	Description	Immature Bone		Mature Bone	
		Absent	Present	Absent	Present
Proximal Cortical Peeling	Peeling of the cortex, involving the proximal half of the bone shaft		Not Observed in Immature Samples		
Distal Cortical Peeling	Peeling of the cortex, involving the distal half of the bone shaft		Not Observed in Immature Samples		
Proximal Metaphyseal Macroscopic Cracking	The cortex of the proximal metaphyseal surface contains at least one crack that is visible to the naked eye		Not Observed in Immature Samples		

Proximal Metaphyseal Microscopic Cracking	The cortex of the proximal metaphyseal surface contains at least one crack that is visible at 10x magnification				
Proximal Metaphyseal Surface Marginal Loss	There is localized loss of the proximal metaphyseal surface involving the perimeter				
Proximal Metaphyseal Surface Central Loss	There is localized loss of the proximal metaphyseal surface that does not involve the perimeter				
Distal Metaphyseal Macroscopic Cracking	The cortex of the distal metaphyseal surface contains at least one crack that is visible to the naked eye		Not Observed in Immature Samples		
Distal Metaphyseal Microscopic Cracking	The cortex of the distal metaphyseal surface contains at least one crack that is visible at 10x magnification				

Distal Metaphyseal Marginal Loss	There is localized loss of the distal metaphyseal surface involving the perimeter				
Distal Metaphyseal Central Loss	There is localized loss of the distal metaphyseal surface that does not involve the perimeter				
Longitudinal Cracking	Cracking of the bone shaft cortex that extends into the trabecular bone and is parallel to the bone grain				Not Observed in Mature Samples

Following their exposure time, all bone samples were macerated in warm water and Tergazyme until all remaining soft tissue had disappeared. The radii were then photographed and observed under a Leica stereomicroscope at 10X magnification. The microscopic traits were recorded under the microscope, while macroscopic traits were evaluated using the naked eye. All changes were recorded as present or absent in each bone specimen and then converted into a frequency of occurrence for each month. Any differences between the weathering patterns of the immature and mature bones, throughout the postmortem interval, were evaluated using a Kolmogorov Smirnov test, which compared the distribution each examined weathering variable between the two maturity groups. The physical changes observed on the surface of the bone through time were also compared to changes in weight loss on ignition results over time using a Kolmogorov Smirnov test, whereby the distribution of each weathering variable was compared to the distribution of each weight loss on ignition component, within each maturity group.

2.5. Results

2.5.1. Weight Loss on Ignition

The weight loss on ignition experiment successfully evaluated the changes in unbound water, bound water, collagen, and mineral content of the immature and mature bone samples through time. The Kruskal Wallis tests, with post-hoc pairwise comparisons compared the similarities in these compositional values between sampling months; these results can be found in the appendices (Appendix A-H) and reveal several trends. The unbound water (Figure 2) underwent a statistically significant decrease between months 6 and 12 in the immature bone samples. The mature samples, on the other hand, have statistically significant local maximums at months 4 and 8, with no overall change between the fresh, and month 12 samples. These changes in unbound water of the immature and mature bone samples, while statistically different, appear to be quite similar (Figure 2).

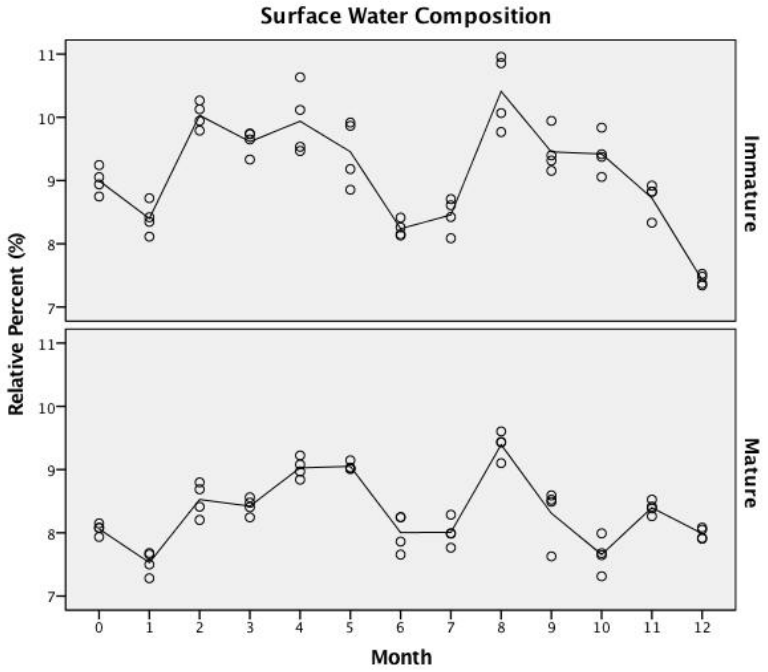


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Figure 2: Changes in unbound water content (% of total weight) over the 12-month interval (n=104). Trend line represents the median values of each month.

The bound water content of the immature bones (Figure 3), exhibited a statistically significant increase from month 2 to 10, then a decrease until the final month of decomposition. In the mature samples, the bound water remained relatively constant throughout the experimental interval, aside from a statistically significant increase from month 3 to 7.

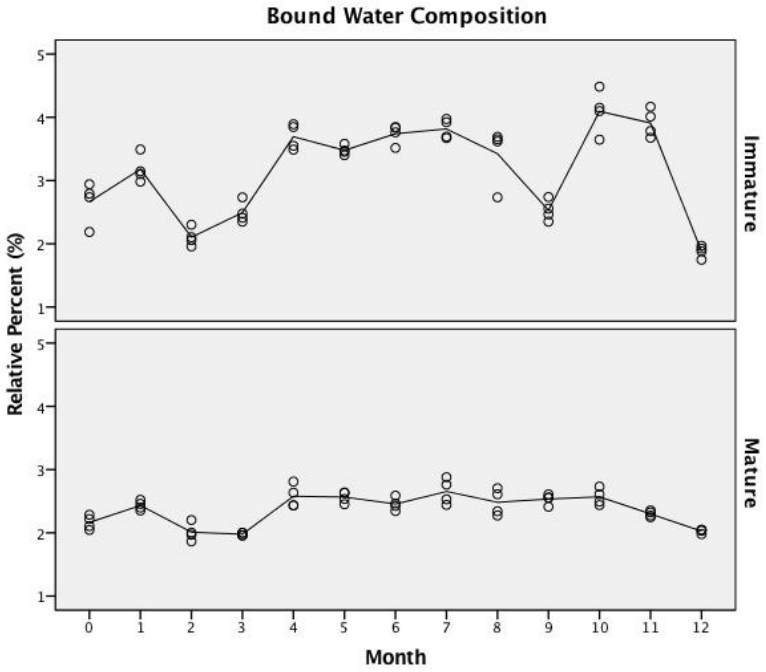

Figure 3: Changes in bound water content (% of total weight) throughout the 12-month interval (n=104). Trend line represents the median values of each month.

The collagen content (Figure 4) increased significantly in the bone samples from both maturity groups; this increase occurred between months 0 to 9 in the immature samples, and between months 0 to 8 in the mature samples. Despite the similar trend between the maturity groups for a majority of the experimental interval- with the immature samples exhibiting more variation- the relative collagen content decreases significantly after reaching a peak at month 9 in the immature samples.

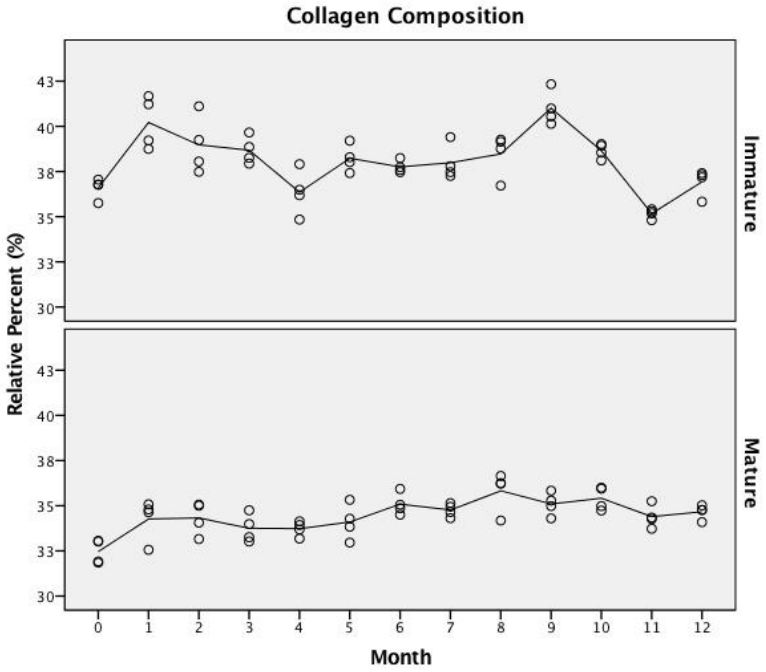

Figure 4: Changes in collagen content (% of total weight) throughout the 12-month interval (n=104). Trend line represents the median values of each month.

Finally, the relative mineral content (Figure 5) showed a statistically significant increase in the immature samples between months 9 to 12. In the mature samples, however, the mineral content experienced a statistically significant, although slight, decrease between months 0 to 8.

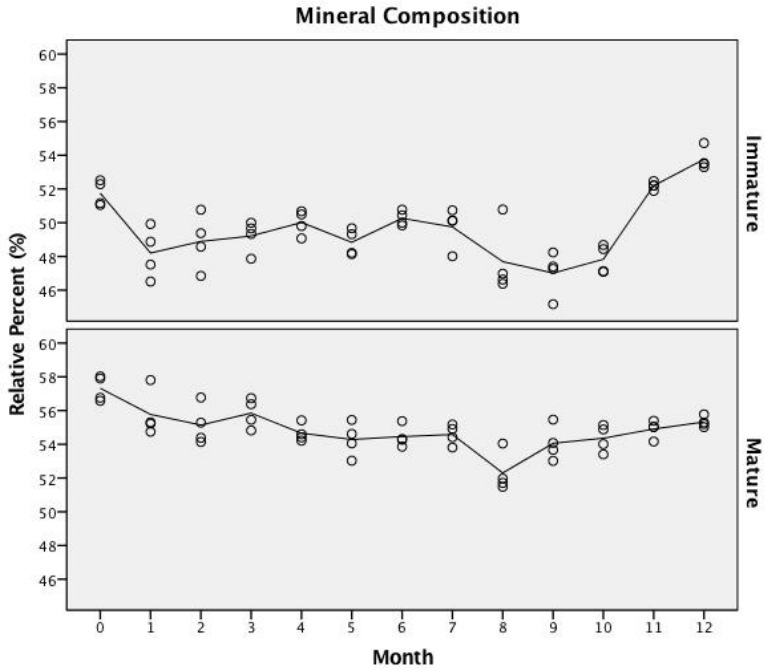

Figure 5: Changes in mineral content (% of total weight) throughout the 12-month interval (n=104). Trend line represents the median values of each month.

The Mann-Whitney results, reported in Table 2, show that the median values of unbound water, bound water, collagen, and mineral differed significantly between the two maturity groups and that these differences were maintained throughout a majority of the experimental interval. Exceptions to this include periodic overlapping values of the unbound water in months 5, 6, 7, and 11; of and bound water quantities in months 1, and 9; as well as the collagen values in the eleventh month of study.

Table 2: Mann-Whitney U test results (z-statistic values for normal approximation are provided) when comparing the medians of each weight loss on ignition variable between immature and mature groups. (*highlights significant values when p<0.05)

Month	*Unbound Water*	*Bound Water*	*Collagen*	*Mineral*
0	Z= -2.309 p= 0.029*	Z= -2.309 p= 0.029*	Z= -2.309 p= 0.029*	Z= -2.309 p= 0.029*
1	Z= -2.309 p= 0.029*	Z= -2.309 p= 0.386	Z= -2.309 p= 0.029*	Z= -2.309 p= 0.029*
2	Z= -2.309 p= 0.029*	Z= -2.309 p= 0.029*	Z= -2.309 p= 0.029*	Z= -2.309 p= 0.029*
3	Z= -2.309 p= 0.029*	Z= -2.309 p= 0.029*	Z= -2.309 p= 0.029*	Z= -2.309 p= 0.029*
4	Z= -2.309 p= 0.029*	Z= -2.309 p= 0.029*	Z= -2.309 p= 0.029*	Z= -2.309 p= 0.029*
5	Z= -1.155 p= 0.343	Z= -2.309 p= 0.029*	Z= -2.309 p= 0.029*	Z= -2.309 p= 0.029*
6	Z= -1.155 p= 0.343	Z= -2.309 p= 0.029*	Z= -2.309 p= 0.029*	Z= -2.309 p= 0.029*
7	Z= -2.021 p= 0.057	Z= -2.309 p= 0.029*	Z= -2.309 p= 0.029*	Z= -2.309 p= 0.029*
8	Z= -2.309 p= 0.029*	Z= -2.309 p= 0.029*	Z= -2.309 p= 0.029*	Z= -2.309 p= 0.029*
9	Z= -2.309 p= 0.029*	Z= -0.145 p= 0.886	Z= -2.309 p= 0.029*	Z= -2.309 p= 0.029*
10	Z= -2.309 p= 0.029*	Z= -2.309 p= 0.029*	Z= -2.309 p= 0.029*	Z= -2.309 p= 0.029*
11	Z= -1.452 p= 0.200	Z= -2.309 p= 0.029*	Z= -1.732 p= 0.114	Z= -2.309 p= 0.029*
12	Z= -2.309 p= 0.029*	Z= -2.309 p= 0.029*	Z= -2.309 p= 0.029*	Z= -2.309 p= 0.029*

The Kolmogorov Smirnov tests comparing the distributions of the unbound water, bound water, collagen, and mineral values between the two maturity groups, through time are given in Table 3. These tests reveal that the distributions of the immature and mature bone components through time are not the same for any of the weight loss on ignition variables, despite the visual similarity observed in the unbound water changes.

This is consistent with the Mann-Whitney U and Kruskal Wallis results, which indicate that there is a difference in both the monthly median values and the way in which they vary through time, between the two analyzed maturity groups.

Table 3: Kolmogorov Smirnov test results when comparing the distributions of each weight loss on ignition variable between immature and mature groups. (*highlights significant values when p<0.05)

Weight Loss on Ignition Variable	*D-Value*	*p- Value*
Unbound Water	2.353	0.000 *
Bound Water	3.334	0.000 *
Collagen	4.216	0.001 *
Mineral	4.413	0.000 *

2.5.2. Bone Surface Weathering Analysis

Observational data (Table 1) revealed that several types of bone breakdown were specific to a single maturity group: proximal and distal peeling appeared only in the mature samples during the first and third months, respectively, and a single immature bone exhibited a longitudinal crack in the final month of analysis. Table 4 shows the results of the Kolmogorov Smirnov tests, where the frequency distributions of the weathering variables were compared between the immature and mature groups over time. Results in this table indicate that there are statistically significant differences between not only the distributions of distal and cortical peeling but also the distributions of metaphyseal plate micro- and macro-cracking between the two maturity groups. These statistical results are consistent with the observation that there was very little cracking in the metaphyseal surface of the immature samples. There was no significant difference between the other quantified variables: longitudinal cracking, which was documented in only one sample, and localized loss the of metaphyseal surfaces, which was observed extensively in both maturity groups.

Table 4: Kolmogorov Smirnov test results when comparing the immature and the mature distributions of each weathering variable over time (*highlights significant values at p<0.05).

Weathering Variable	*D- Value*	*p- Value*
Proximal Cortical Peeling	2.353	**0.000 ***
Distal Cortical Peeling	1.961	**0.001 ***
Longitudinal Cracking	0.196	1.000
Proximal Metaphyseal Macroscopic Cracking	1.961	**0.001 ***
Proximal Metaphyseal Microscopic Cracking	2.353	**0.000 ***
Proximal Metaphyseal Marginal Loss	1.177	0.125
Proximal Metaphyseal Central Loss	0.588	0.879
Distal Metaphyseal Macroscopic Cracking	2.157	**0.000 ***
Distal Metaphyseal Microscopic Cracking	1.961	**0.001 ***
Distal Metaphyseal Marginal Loss	0.392	0.998
Distal Metaphyseal Central Loss	0.784	0.570

When comparing the compositional changes in the bone material with the physical changes, Kolmogorov Smirnov tests showed that in the mature bone samples (Table 5), the distribution of all compositional variables differed in a statistically significant manner from the distribution of the weathering variables. In the immature samples (Table 6), however, the distribution of the mineral content through time did not differ significantly from the observed frequencies of proximal and distal metaphyseal surface loss in the central region. Further, the distribution of the collagen content did not differ significantly from the central loss of the proximal metaphyseal surface. This suggests that an increase in the relative mineral composition, with resulting decrease in relative collagen content, of immature bones is associated with increasing localized loss of metaphyseal surface bone, within a subaerial environment.

Table 5: Kolmogorov Smirnov test results when comparing the distributions of each weight loss on ignition variable to the measured weathering variables in the immature bone sample. (*highlights distributions that <u>do not</u> differ significantly at p>0.05)

Weathering Variable	*Unbound Water*	*Bound Water*	*Collagen*	*Mineral*
Proximal Cortical Peeling	D= 1.000 p= 0.000	D= 1.000 p= 0.000	D= 1.000 p= 0.000	D= 1.000 p= 0.000
Distal Cortical Peeling	D= 0.917 p= 0.000	D= 0.917 p= 0.000	D= 1.000 p= 0.000	D= 1.000 p= 0.000
Longitudinal Cracking	D= 0.917 p= 0.000	D= 0.917 p= 0.000	D= 1.000 p= 0.000	D= 1.000 p= 0.000
Proximal Metaphyseal Macroscopic Cracking	D= 1.000 p= 0.000	D= 1.000 p= 0.000	D= 1.000 p= 0.000	D= 1.000 p= 0.000
Proximal Metaphyseal Microscopic Cracking	D= 0.833 p= 0.000	D= 0.833 p= 0.000	D= 0.917 p= 0.000	D= 0.917 p= 0.000
Proximal Metaphyseal Marginal Loss	D= 0.917 p= 0.000	D= 0.917 p= 0.000	D= 0.917 p= 0.000	D= 0.833 p= 0.000
Proximal Metaphyseal Central Loss	D= 0.667 p= 0.008	D= 0.667 p= 0.008	**D= 0.500 p= 0.088 ***	**D= 0.500 p= 0.088 ***
Distal Metaphyseal Macroscopic Cracking	D= 0.833 p= 0.000	D= 0.833 p= 0.000	D= 1.000 p= 0.000	D= 1.000 p= 0.000
Distal Metaphyseal Microscopic Cracking	D= 0.667 p= 0.008	D= 0.667 p= 0.008	D= 0.593 p= 0.029	D= 0.593 p= 0.029
Distal Metaphyseal Marginal Loss	D= 1.000 p= 0.000	D= 1.000 p= 0.000	D= 1.000 p= 0.000	D= 0.833 p= 0.000
Distal Metaphyseal Central Loss	D= 0.917 p= 0.000	D= 0.917 p= 0.000	D= 0.667 p= 0.008	**D= 0.333 p= 0.492 ***

Table 6: Kolmogorov Smirnov test results when comparing the distributions of each weight loss on ignition variable to the measured weathering variables in the mature bone sample. (*highlights distributions that <u>do not</u> differ significantly at p>0.05)

Weathering Variable	*Unbound Water*	*Bound Water*	*Collagen*	*Mineral*
Proximal Cortical Peeling	D= 0.769 p= 0.001	D= 0.769 p= 0.001	D= 0.769 p= 0.001	D= 0.769 p= 0.001
Distal Cortical Peeling	D= 0.923 p= 0.000	D= 0.923 p= 0.000	D= 0.923 p= 0.000	D= 0.846 p= 0.000
Longitudinal Cracking	D= 1.000 p= 0.000	D= 1.000 p= 0.000	D= 1.000 p= 0.000	D= 1.000 p= 0.000
Proximal Metaphyseal Macroscopic Cracking	D= 0.769 p= 0.001	D= 0.769 p= 0.001	D= 0.615 p= 0.015	D= 0.538 p= 0.046
Proximal Metaphyseal Microscopic Cracking	D= 1.000 p= 0.000	D= 1.000 p= 0.000	D= 0.923 p= 0.000	D= 0.923 p= 0.000
Proximal Metaphyseal Marginal Loss	D= 0.923 p= 0.000	D= 0.923 p= 0.000	D= 0.615 p= 0.015	D= 0.692 p= 0.004
Proximal Metaphyseal Central Loss	D= 0.692 p= 0.004	D= 0.692 p= 0.004	D= 0.692 p= 0.004	D= 0.846 p= 0.000
Distal Metaphyseal Macroscopic Cracking	D= 0.846 p= 0.000	D= 0.846 p= 0.000	D= 0.846 p= 0.000	D= 0.769 p= 0.001
Distal Metaphyseal Microscopic Cracking	D= 0.923 p= 0.000	D= 0.923 p= 0.000	D= 0.923 p= 0.000	D= 0.923 p= 0.000
Distal Metaphyseal Marginal Loss	D= 1.000 p= 0.000	D= 1.000 p= 0.000	D= 1.000 p= 0.000	D= 0.769 p= 0.001
Distal Metaphyseal Central Loss	D= 1.000 p= 0.000	D= 1.000 p= 0.000	D= 0.769 p= 0.001	D= 0.615 p= 0.015

2.6. Discussion

2.6.1. Weight Loss on Ignition

The results of this study are consistent with the current literature pertaining to bone composition. The immature samples contained a higher collagen and water content, and a lower mineral content than the mature bone samples, and this difference was maintained through time. The relative percent composition of collagen was approximately 39% in the immature bone, and 35% in the mature bone. The relative percent composition of mineral was approximately 47% in the immature samples, and 54% in the mature samples. While these values are inconsistent with the normative values of human bone, with approximately 10% being water (Manilay 2013), 20-25% being organic collagen (Buckberry 2000, Hillier and Bell 2007, Manilay 2013, Tappen 1994), and 60-70% being mineral (Boskey 2014, Manilay 2013), they are somewhat similar to the values obtained from porcine femora of varying ages. Weight loss on ignition analysis performed by Chittenden et al. (2015) determined that the relative collagen content of porcine bone is close to 30% in pigs aged 1 month (the age of our immature samples), but decreases significantly by 6 months of age (the age of our mature samples), and that the mineral content increases from approximately 35% at 1 month, to 60% at 6 months of age. Studies such as this further support the body of evidence for the dependence of bone composition on maturity.

In addition to absolute differences in weight loss on ignition values, the changes in relative unbound water, bound water, collagen, and mineral content through time, observed in this study, also differed significantly between the immature and mature bone samples. The relative unbound and bound water contents of the immature bones was extremely variable through time, while the mature bones exhibited a comparatively constant hydration level. The relative collagen content decreased, and the mineral content increased significantly in the immature samples within the final months of the experiment, while the mature samples demonstrated no such change. These differences may be attributed to the maturity dependency of porosity in skeletal material, with immature bones containing more vascularized macroporosity (Manifold 2014), and an extensive collagen network that exposes microporosity when lost (Hedges 1995b). The size and distribution of holes within a bone dictate the interactions between the osseous material and its environment (Hedges and Millard 1995a, 1995b). The intrinsic feature of

high bone porosity in less mature bone material therefore provides an avenue for more rapid infiltration of the bone by external degradation factors such as moisture, or microorganisms (Hedges and Millard 1995a, Jans et al. 2004, Manifold 2012), as well as a larger internal and external surface area over which the integrity of the bone may be attacked (Boaks et al. 2014, Buckberry 2000, Djuric et al. 2011, Garland 1987, Hedges and Millard 1995a, Jans et al. 2004, Lewis 2007, Manifold 2012, Mays 2010). This fact is exacerbated by the maturity-dependent structure of bone mineral scaffolding. The arrangement of the hydroxide and carbonate ions, within the mineral lattice of bone, begins as a scaffold composed of tiny crystals (Guy 1987). With increasing maturity, the bone takes up ions, such as fluoride (Guy 1987, Weiner and Wagner 1998), and increase the size of its mineral crystals to become more thermodynamically stable (Guy 1987, Hedges and Millard 1995b, Mays 2010). These changes in conformation are reflected in the increasing relative mineral composition of the bone, as well as its decreasing porosity with maturity (Guy 1987). As a result of these maturity-dependent changes, external factors such as bacteria that consume the collagen fibrils within bone (Jans et al. 2004), would have had a heightened ability to move through the interior regions of a less mature bone.

The compositional analyses performed in this study suggest that within the early postmortem period in a subaerial degradation environment, the relative composition of bone and its changes through time are dependent upon the maturity of the skeletal material. The significant decrease in relative collagen and increase in mineral content that occurred in the immature samples, during the final 3 months of the experimental interval, suggest that the organic component of the immature samples may be degrading faster than that of the mature bone samples. Bone weathering analysis was included to address whether these differences affected the integrity of the bone.

2.6.2. Bone Surface Weathering

The bone weathering observations indicate that physical changes of bone material, relative to its environment, are also dependent upon the maturity of the bone. The outer cortex of the mature bone samples demonstrated peeling along the entirety of the shaft after the first month, while no peeling was found in the immature samples. Further, the presence of metaphyseal cracking was statistically more frequent in the mature bone samples, beginning in the first month. This indicates that the mature bone

samples may have been more susceptible to physical degradation than the immature bones.

The observed differences in the types of physical bone surface breakdown in each maturity group can be explained by developmental differences in the structure of the bone material. Long bone forms through a process known as endochondral ossification. An initial cartilaginous precursor is replaced by osseous tissue, as it is laid down in concentric rings to create osteons. These osteons surround a series of Haversian canals that provide a route for vasculature and nerves within the bone (Hillier and Bell 2007, Manifold 2014, Weiner and Wagner 1998). During the initial stages of ossification, the skeleton of a neonate is arranged with longitudinal, radial, and circumferential osteons; this is known as plexiform bone and it is found in both porcine bone and immature human bone (Hillier and Bell 2007). As the skeleton matures, the cortex becomes thicker and some regions of plexiform bone are replaced by lamellar bone (Manilay et al. 2013), in which the Haversian systems and accompanying concentric bone layers run longitudinally through the bone (Hillier and Bell 2007). The mature lamellar bone becomes increasingly resistant to compressive forces; however, it loses its elasticity as the original collagen content is replaced by a larger, parallel, mineral structure (Guy 1987). The immature and mature bones used in this study contain varying quantities of plexiform bone, as lamellar bone is incorporated into the structure of the more mature bones (Hillier and Bell 2007). As these samples were exposed to ultra-violet radiation, the collagen within their osseous scaffolds may have been degraded by photolytic and photo-oxidative reactions (Dupras and Schultz 2012, Zayat et al. 2007). A loss of protein results in brittle bone material that is susceptible to cracking and flaking (Dupras and Schultz 2012, Junod and Pokines 2012). If these processes were occurring within the studied bone samples, then the outer layers may have been modified by sunlight first. In a mature bone sample, with a higher degree of lamellar structure, the outer concentric layers could then easily be separated from one another to result in cortical cracking and peeling. In an immature plexiform bone, however, the multi-directionality of the structure and the highly elastic collagen component could prevent any brittle cracking or organized peeling of the outer layers of bone.

2.6.3. The Role of Compositional Change in Physical Destruction

The combination of the weight loss on ignition results and the bone weathering results suggests that, within the early postmortem interval of subaerial bone degradation, maturity plays a determining role in the type of changes observed. Mature bone was found to be more susceptible to physical surface weathering by its environment, while immature bone underwent more pronounced compositional changes. Further, the changes in the mineral component of the immature bone samples were found to be associated with localized loss in the center of both metaphyseal surfaces. If causative, this relationship can most easily be explained by the relative increase in mineral content, with the corresponding decrease in collagen that was observed in the immature bones in the final 3 months of analysis. With the measured loss of collagen, the bones would have become increasingly brittle, making them prone to cracking and breakage (Dupras and Schultz 2012, Junod and Pokines 2012), especially within the delicate surface of the metaphyses (Djuric et al. 2011, Lewis 2007). This same localized loss within the metaphyseal surface, however, was determined to be equal in its distribution through time between the two maturity groups; this is most likely due to their structural differences. The plexiform bone structure could have prevented further destruction, despite the compositional changes that were found to be associated with their physical weathering.

2.6.4. Limitations of this Study

This study provides a controlled comparison of the compositional and physical breakdown of immature and mature bone material. While this study is valuable to informing the forensic determination of postmortem interval in juveniles, it does not attempt to create a scale with which this timeframe can be estimated. The mechanical removal of flesh eliminated a critical part of the degradation environment, from which autolysis and microbial attack normally stem (Bilheux et al. 2015, Donaldson and Lamont 2014, Ross and Cunningham 2011). The removal of flesh did, however, ensure that differential soft tissue decomposition between immature and mature individuals could not affect bone weathering, as all other factors were controlled. This maceration protocol, along with the setup, weight loss on ignition, and bone weathering protocols, provides the limiting factors involved in this study

The experiment was carried out in a monitored environment to ensure that all bone samples experienced equivalent external conditions at any given time. While the depositional environment was held constant across all experimental plots, it was not held constant through time. This variation may have resulted in a non-linear accumulation of degradative effects, which would have caused the rate of degradation to slow or accelerate at times. Any plateaus in degradation would prevent normalization of the breakdown rate per unit time, but this was mediated by prevention of large fluctuations in temperature and moisture. Further, the conditions of this experiment were more representative of the naturally occurring ultra violet light and temperature exposure within a subaerial deposition environment.

The weight loss on ignition analysis necessitated the destruction of a section from each sample, which increased the number of bone samples needed. Given that a new set of samples was required for each month of analysis, the compositional and physical analyses were not run on the same bones throughout the year-long study interval. Individual variation could, therefore, have masked trends that would have been noticed had a single set of samples been observed for the full postmortem interval. The time required for mechanical defleshing dictated the number of samples that could be prepared, which in turn caused the problem of sample size. With only 4 samples per maturity group, the individual variation and outliers could have caused more extreme fluctuations in weight loss on ignition values through time. This problem was addressed by using the median values of the samples, for each month. Finally, the furnace used for this weight loss on ignition protocol was often inaccurate at temperatures below 100°C. Fluctuations in the temperature of the furnace may have affected the distinction between unbound and bound water if some bound water was included in the unbound water mass.

The bone weathering protocol quantified only the changes that were observed in the bone samples. These small-scale bone surface physical changes have not been extensively documented in any short-term taphonomy studies, such as those performed by Cunningham et al. (2011), or Janjua and Rogers (2008), and are unlike the variables considered in the well-known stages laid out by Behrensmeyer in 1978. The bone weathering protocol was created to address bone changes occurring in a relatively short period of time, in a single bony element, and in an experimental study that exposed bone to its depositional environment without the soft tissue. Although this protocol is unlike the

larger scale weathering protocols used in other studies, they are useful in documenting changes in the early postmortem interval at a finer scale and higher resolution. Furthermore, they were designed specifically to examine maturity-dependent changes and may be explained by maturity-dependent differences in bone structure.

Despite the limitations imposed by the protocols used in this study, the methods were extremely cost and time effective, and well-suited for the necessary analyses. Careful consideration of the equipment, samples, and experimental time frame allowed for the problems to be addressed and minimized as much as possible.

2.6.5. Significance

This project provides a novel comparison of the compositional changes and surface alteration of immature and mature bone material in a subaerial environment. While past studies have attempted to quantify taphonomic bone changes within a single maturity level, and at the scale of a whole skeleton, the comparative literature on immature and mature bone breakdown is extremely lacking. The results of this study indicate that, within the early postmortem interval of a subaerial degradation environment, maturity can play a significant role in determining the type of alteration observed in bone samples. This suggests that the current methods in the forensic estimation of postmortem interval, which were informed by studies performed on adults, may be seriously compromised. Integration of this new, comparative information into the field of forensic research will help to improve the accuracy with which time-since-death of juveniles is determined.

The results also suggest that taphonomic interpretations in ecological and paleontological studies of immature bone material should be done so with caution, as the body of past research is lacking in this area. The current study addressed only the early postmortem changes of immature bone in comparison to the breakdown of mature bone in an identical environment. Previous studies that examine immature bone weathering either do not do so in a comparative nature, such as Cunngingham et al. (2011) and Janjua and Rogers (2008), or are examining gross changes in whole bones or complete skeletons. Therefore, it is not known how small differences observed in the early stages of bone weathering affect the long-term survival of immature bone material. The results of long-term studies, such as that of Behrensmeyer (1978), who addressed

changes in bone material over a span of 15 years, and Andrews (1995), who examined the first 5 years of postmortem change, documented observational data pertaining to extensive bone cracking and fragmentation, the likes of which were not observed in any short-term studies. If maturity-dependent intrinsic bone qualities are causative of differential bone weathering over an extended postmortem interval, then the current bone weathering indices must be used with caution when assessing paleontological immature bone material.

2.7. Conclusion

Within the early postmortem interval of a subaerial environment, the compositional changes and surface alterations that occur in bone material vary with bone maturity. This is consistent with, not only the hypothesis that maturity-dependent intrinsic bone qualities should affect their weathering, but also with the preliminary results of Gonzales et al (2011), which stated that bone of varying maturities will degrade differently. The present study suggests that immature bone changes primarily in composition early on, then is affected by physical breakdown later on, whereas mature bone experiences physical changes early, while the gross composition is largely unchanged within the first year. Further, the mineral compositional changes observed in immature bone were indicated as being associated with central loss of the metaphyseal surfaces, however, it was not found to cause a more rapid breakdown of gross structure than in the mature bone counterparts. These results indicate that maturity mediates the way in which a bone degrades, however, they cannot address the speed to which bone destruction will occur in a forensic context. Further research into defining how the initial maturity-dependent differences in breakdown translate into the longer-term survival of immature bone material is integral to the process by which time-since-death is estimated and skeletonized juveniles remains are identified.

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Chapter 3.

Differential Weathering of Immature and Mature Bone in a Buried Environment

This chapter will be submitted for publication in the Journal of Archaeological Sciences.

3.1. Abstract

In addition to differential culture-specific burial practices, the relative absence of juvenile skeletal remains in historic cemetery excavations has been explained by a comparatively rapid breakdown of immature bone. While the idea of differing breakdown rates between immature and mature bone is widely accepted, few experiments have provided evidence to support it.

This study uses a porcine model to explore the role of bone maturity with regards to the overall susceptibility of the skeleton to physical and compositional degradation in a buried environment. Samples (n=104) were mechanically defleshed and left to degrade in a climate-controlled greenhouse environment, buried 10cm below the soil surface. Every month, for the span of 12 months, 4 immature and mature bones were collected. Weight loss on ignition analysis was performed to determine the composition of the bones, and bone weathering analysis was carried out to quantify the physical breakdown of the bones.

The results of this study indicate that in the early postmortem interval of buried skeletal remains the compositional and physical breakdown of immature bones differ significantly from the changes seen in their mature bone counterparts. Immature bones are more susceptible to compositional changes, but less affected by physical surface weathering. How these early differences in degradation affect the long-term survival of bone material and its post-depositional history in archaeological contexts, however, requires a longer study interval.

HIGHLIGHTS: (3-5 85 max characters each)

- Immature and mature pig bones are used as a proxy for the human skeleton

- A controlled burial environment allows for a direct comparison of degradation
- Weight loss on ignition analysis determines that compositional breakdown is dependent on maturity of bone
- Bone weathering analysis determines that physical decay is dependent on maturity of bone

3.2. Keywords (3-7):

Osteology, Immature Bone, Taphonomy, Bone Chemistry, Bone Weathering

3.3. Introduction

Juvenile remains are often significantly underrepresented in archaeological cemetery excavations (Bello and Andrews 2006, Buckberry 2000, Djuric et al. 2011, Guy 1997, Lewis 2007, Manifold 2010, 2012, 2013, Mays 2010, Walker et al. 1988). Based on the idea that archaeological societies should be comparable to pre-industrialized ones, it is expected that about 30% of cemetery remains should be children, but this is rarely the case (Akazawa et al. 1995, Lewis 2007, Saunders 2008). This confounding paucity in the archaeological record causes problems for paleodemographic and bioarchaeological studies, which require that a skeletal sample be representative of the living population (Angel 1969, Bello and Andrews 2006, Djuric et al. 2011, Lovejoy 1971, Manifold 2010, Roksandic and Armstrong 2011, Walker et al. 1988, Wood et al. 1992). While researchers recognize and attempt to compensate for this deviation from expected population structures, no single explanation can account for its widespread prevalence (Bello and Andrews 2006, Guy 1997, Lewis 2007).

It is generally accepted that taphonomic processes act most heavily upon juvenile remains, due to their smaller size, incomplete mineralization, higher organic and water content, and higher porosity than their adult counterparts (Bello and Andrews 2006, Buckberry 2000, Djuric et al. 2011, Gordon and Buikstra 1981, Guy 1997, Lewis 2007, Manifold 2010, 2012, 2013, Mays 2010, Walker et al. 1988). The magnitude to which these intrinsic factors affect bone degradation, however, is still not well understood (Djuric et al. 2011, Manifold 2010, Walker et al. 1988). While many archaeologists favor the idea of rapid juvenile bone destruction, regardless of the lack of supporting evidence, variable preservation of archaeological juvenile skeletal material suggests that there are many other factors at play (Bello and Andrews 2006, Buckberry

2000, Djuric et al. 2011, Gordon and Buikstra 1981, Guy 1997, Lewis 2007, Manifold 2010, 2012, 2013, Mays 2010, Walker et al. 1988). The preserved remains of a 100000 year old Neanderthal child (Akazawa et al. 1995), and the excellent preservation of juveniles within a 19th century Californian cemetery (Buckberry 2000) are just a few examples of what caused archaeologists to reconsider the situation. The other cause being put forward to explain the paucity of juvenile remains in archaeological samples is the differential treatment of children burials in past cultures (Bello and Andrews 2006, Buckberry 2000, Guy 1997, Lewis 2007, Mays 2010, Manifold 2010, 2012, 2013). In many cultures, children were not buried in the same location as the adults, making their retrieval difficult without prior knowledge of these practices. For example, in 17th century English Catholic societies neonates could not be buried on consecrated cemetery grounds unless a baptism and funeral were paid for, resulting in their burial along the outside of cemetery walls (Guy 1997, Lewis 2007). A third, and often overlooked, explanation for the paucity of juvenile remains is the modern archaeological techniques that are not often tailored towards retrieving juvenile remains (Buckberry 2000, Lewis 2007, Mays 2010, Manifold 2010, 2012, 2013, Saunders 2008). Immature skeletal remains are composed of more bones that are smaller, more irregularly shaped, and not often outlined on archaeological recovery sheets, resulting in poor recognition and recovery of these bones (Lewis 2007, Manifold 2010, Saunders 2008). Further, rough handling and screening techniques risk loss and destruction of these small, fragile elements (Buckberry 2000, Henderson 1987, Lewis 2007, Manifold 2012, Mays 2010). Although evidence for differential burial treatment in past societies, as well as the current methods in archaeological excavation, help to provide explanation for the lack of juvenile remains in archaeological contexts, differential preservation between adult and non-adult skeletal remains cannot be supported nor refuted without experimental evidence.

Weathering is the first stage by which bone starts to compositionally and physically degrade once it has been exposed to its burial or deposition environment. The first formal definition of bone weathering, put forward by Behrensmeyer (1978), stated that it is the process of separating and destroying the microscopic structure of the organic and inorganic components of bone. This research went on to provide the first index for quantifying the effects of the deposition environment on physical breakdown of bone material (Behrensmeyer 1978, Lyman and Fox 1989, Ubelaker 1997). While widely accepted and utilized by taphonomists and zooarchaeologists, this index produced

stages that not only introduce discontinuity into a continuous process (Lyman and Fox 1989), but also are specific to large adult mammals, and provide non-specific time spans (Haglund and Sorg 1997, Madgewick and Mulville 2012, Tappen 1994). Since, researchers have attempted to refine the Behrensmeyer weathering index by including new postmortem environments, different human analogs, and observations of human archaeological skeletons. Andrews and Cook (1985), for example, compiled observational data on a bovine skeleton over the course of 7.5 years in order to model the breakdown of undisturbed bone material. Later, Andrews (1990) studied small animal remains within owl pellets and developed a scale with which to estimate the depositional period of smaller remains. Mckinley (2004) attempted to make weathering more applicable to the human skeleton by developing a weathering scale based on archaeological assemblages. Many other studies have attempted to further this research by studying the breakdown of mature bone, while stating that immature bone may degrade faster. Despite this assertion, the existing research on immature skeletal breakdown is extremely lacking. In 2011 Cunningham et al. experimentally observed the breakdown of immature pig bones over the course of 11 months. This study used protected, fleshed pig cadavers for the purpose of developing a bone weathering index that is applicable to the early postmortem interval. Janjua and Rogers (2008) also used immature pig remains to study the early postmortem interval, but they carried out a comparison between fleshed and defleshed samples. Gonzalez et al. (2011), unlike the other studies of immature bone material, designed a comparison of the breakdown rates of guanaco bones from three distinct maturity groups. This study suggested that the maturity of an individual aids in determining the susceptibility of their bone material to breakdown (Gonzalez et al. 2011, Gutierrez et al. 2010). Studies such as this, despite small sample size and only preliminary results, act as a starting point for further research on the differential breakdown of immature and mature bone material.

This project was designed to contribute to the archaeological knowledge about the physical and compositional degradation rates of buried immature and mature bone within the early postmortem period. The maturity-dependent intrinsic differences of bone material were evaluated as being associated with the differential breakdown rates of bone from varying maturity levels. This was achieved through the compositional and observational analysis of pig bone, acting as a proxy for the human skeleton, within the first year of degradation in a controlled burial environment. The hypothesis tested was: *if*

maturity-dependent intrinsic qualities of bone have an effect on the breakdown rate of the material, then in the same depositional environment the immature and mature bone will exhibit differential bone breakdown responses. The results from this study can be applied to the questions surrounding juvenile bone survival in the archaeological record.

3.4. Materials and Methods

3.4.1. The Experimental Setup

This experiment was designed to directly compare the effects of a consistent buried environment on the early stages of immature and mature bone weathering, using pig (*Sus scrofa*) remains. Pigs were chosen as a proxy for humans as they are regularly used in degradation experiments, given the biological and morphological similarity of their skeletons, and their commercial availability (Cunningham et al. 2011, Turner and Wiltshire 1999). Only the forelimbs of the pigs were used for this project in order to control for intrinsic factors in bone weathering, including size, shape, surface area, and bone density. The bone samples were obtained from a local meat supplier as fleshed forelimbs from pigs of two distinct maturity groups: immature aged between 2-8 weeks (n=52), and mature aged at 6 months (n=52). The samples were prepared by mechanically defleshing and extracting the radius and ulna from each forelimb, weighing and measuring them, then storing them in labeled bags at 4°C until being brought to the deposition site.

The depositional environment used in this study was confined to a climate-controlled greenhouse, allowing extrinsic factors to be closely monitored across all experimental plots. These plots were prepared by layering 10cm of homogenized organic soil and sand in 60x40x32cm (53L) Rubbermaid® containers with drainage holes. The bone samples were then buried at a 10cm depth. Twelve containers were created to provide an experimental plot for each month of the 1-year study period. The plots contained 4 bones from each maturity group that were exposed to the same external factors throughout the duration of the experiment.

Monitoring and control over extrinsic factors were performed on a weekly basis to ensure that the atmospheric temperature and humidity of the greenhouse did not vary immensely with the seasons, and that the pH, moisture content, and temperature of the

soil did not vary between the different experimental plots. These measurements were obtained using an ambient measuring device in the greenhouse, and a portable pH, moisture, and temperature probe 10cm deep in the soil. Fluctuations in soil moisture were mitigated with regular watering.

Every 30 days, for the span of one year, the bones from one Rubbermaid container were excavated and collected for analysis. The 4 bones from each maturity group were collected, freed of excess dirt, then transferred into polyethylene bags. The bagged samples were then photographed and stored at 4°C for the rest of the experimental interval to prevent bacterial growth (Micozzi 1997).

3.4.2. Weight Loss on Ignition

This protocol was designed as an adaptation of previous bone ashing experiments, such as those by Nielsen et al. (1980), Lochmuller et al. (2000), Park et al. (2003), and Pienkowski et al. (2009), in order to detect any compositional breakdown of the bone samples during the one year study period. The methods employed by Lochmuller et al. (2000) were validated using Dual-Energy X-Ray Absorptiometry, while then results of Pienkowski et al. (2009) were validated using Fourier Transform Infrared Spectroscopy. This supports the effectiveness of this protocol to determine the unbound water, bound water, collagen, and mineral quantities in bone material using a cost-effective and relatively simple method of weight of loss on ignition analysis (Fisk et al. 2017).

The ulna of each forelimb in the immature and mature groups was sacrificed to obtain a bone sample of at least $1cm^3$ from the shaft, immediately following collection from their depositional environment. Samples were agitated in a 2:1 mixture of chloroform and methanol for 24 hours to remove fat and adhered tissues. The samples were then heated at room temperature, 65°C, 105°C, and 600°C, and weighed between each increase in temperature. The temperatures were adapted from the aforementioned previous bone ignition studies, and optimized to our sample size and timeframe in order to measure the relative unbound water, bound water, collagen, and mineral content by weight. The steps are given in Figure 6.

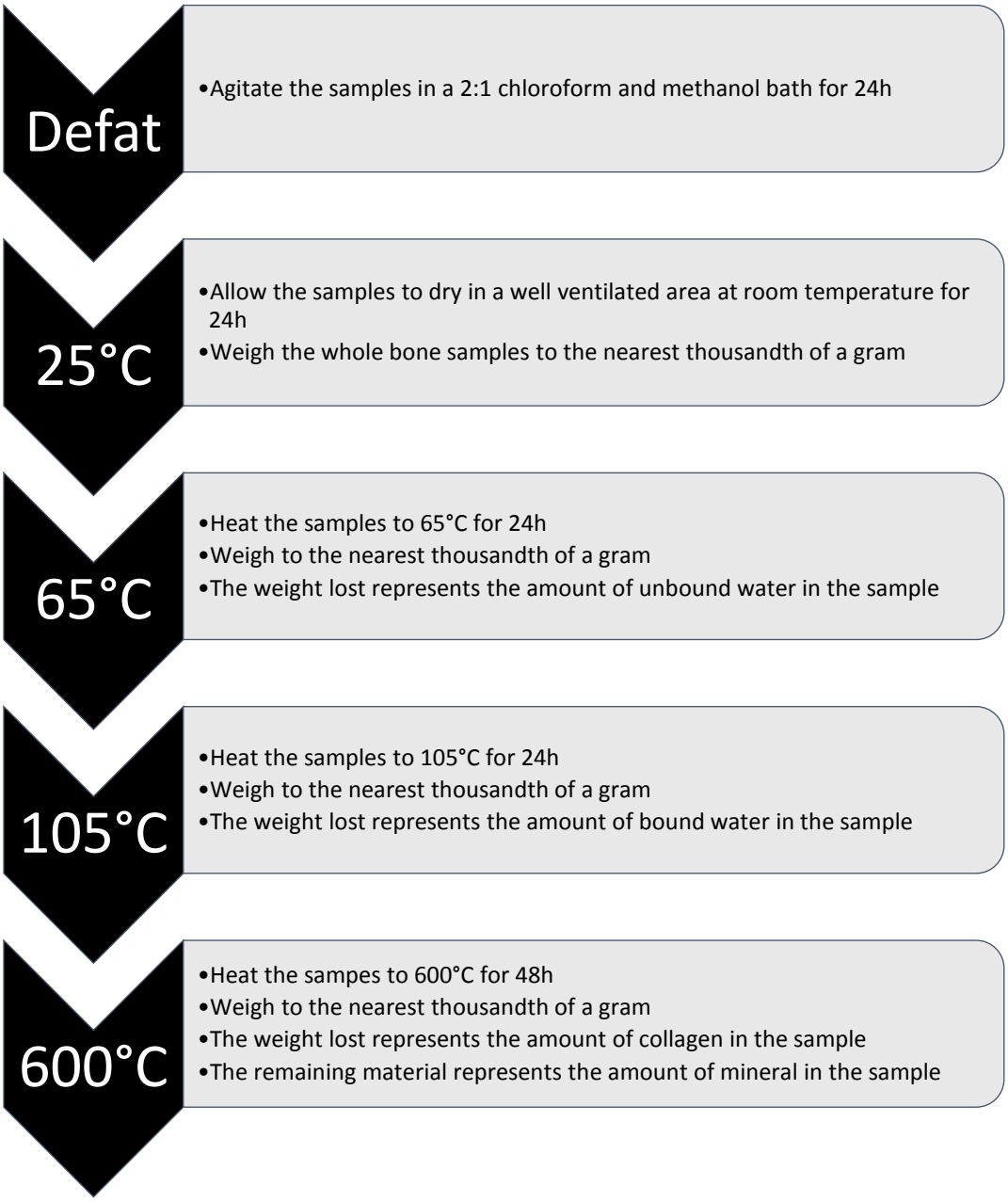


Figure 6: Steps in the weight loss on ignition protocol.

A Kuskal Wallis and post-hoc pairwise comparison, with Bonferroni correction, were used to analyze the changes in the relative proportions of unbound water, bound water, collagen, and mineral throughout the experimental interval, for each maturity group. This provided an in-depth analysis of how the bone composition changed during each month, within each maturity group. A Mann Whitney U test was then used to

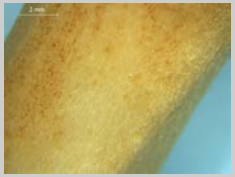
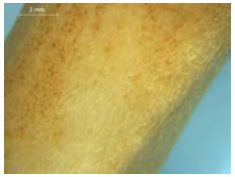
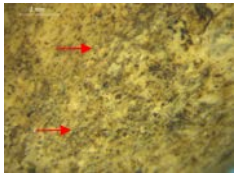
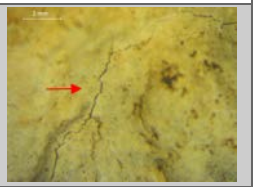
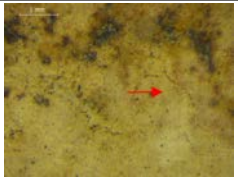
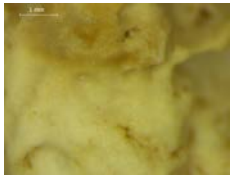
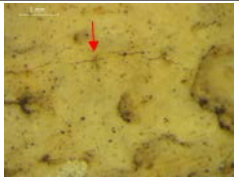
compare the relative proportions of unbound water, bound water, collagen, and mineral between the varying maturity groups for each month. Additionally, a Kolmogorov Smirnov test was used to determine if the distribution of each weight loss on ignition variable through time differed significantly between the two maturity groups. These statistical tests allowed comparison of the values of each bone component in order to evaluate the intrinsic differences in composition due to maturity, as well as if these differences were maintained through time.

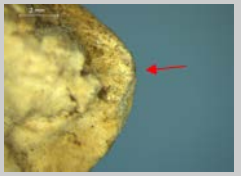
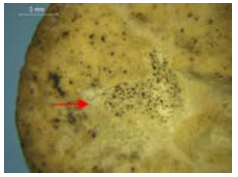
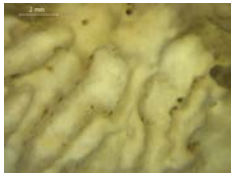
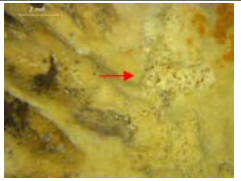
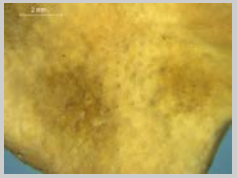
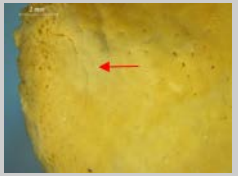
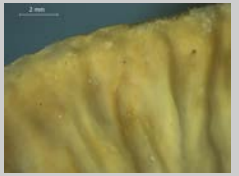
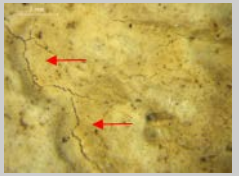
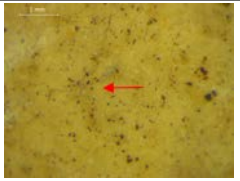
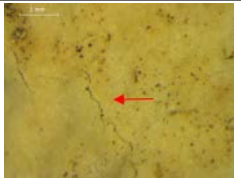
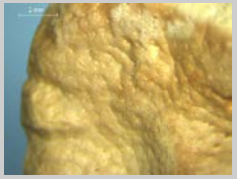
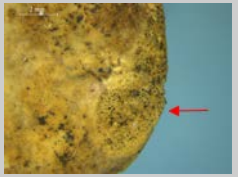
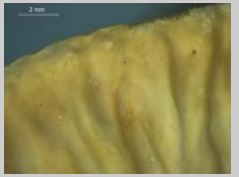
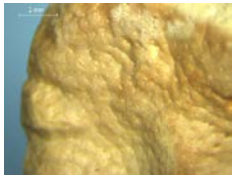
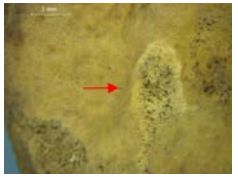
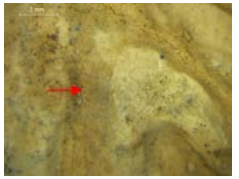
3.4.3. Bone Surface Weathering Analysis

The bone weathering analysis consisted of visually examining the physical changes on the surface of the immature and mature radii samples that occurred over time. After examining all of the bone samples under a Leica stereomicroscope at 10x magnification, a series of surface alterations were identified and categorized to reflect their impact on the superficial bone structure. The variables considered were similar to those in the literature, such as the mosaic cracking observed by Behrensmeyer (1978), as well the localized cortical loss referred to as ‘pockmarking’ and the cortical roughness termed ‘marbling’ by Cunngingham and Ross (2011). The selected weathering changes were included based on their presence in at least one bone sample, and were further split into sub-categories when the affected regions of bone differed through time.

The 10 variables that were examined include cortical roughness, cortical peeling, and cracking and loss of the metaphyseal surfaces. Cortical roughness was characterized by destruction of the smooth outer cortex, and was classified as linear when the bone grain was maintained, or multidirectional when it was not. Cortical peeling was observed as the lifting and separating of the outermost layer of the bone diaphysis, and was categorized by the region of bone that it affected. Cracking of the metaphyseal surface was denoted by the presence of at least one crack, that was further classified as macroscopic (visible to the naked eye), or microscopic (visible at 10x magnification). Localized loss of the metaphyseal surfaces was considered as the loss of cortical bone to reveal the underlying trabeculae in either the peripheral or central regions of the metaphyseal surfaces. A complete list of the weathering variables used in this project, as well as their illustrations, are given in Table 7.

Table 7: **The bone surface weathering variables, their descriptions, and illustrations of their absence and presence. All images depicting the absence of a trait were obtained from fresh bone samples, while those with surface alterations were obtained from bones that decayed in a buried environment. Arrows indicate features present. Magnification 10x-20x.**

Variable	Description	Immature Bone		Mature Bone	
		Absent	Present	Absent	Present
Linear Cortical Roughness	The surface of the bone shaft is no longer smooth, while the grain of the bone is maintained				
Multidirectional Cortical Roughness	The surface of the bone shaft is no longer smooth and the grain of the bone is no longer present		Not Observed in Immature Samples		
Proximal Metaphyseal Macroscopic Cracking	The cortex of the proximal metaphyseal surface contains at least one crack that is visible to the naked eye		Not Observed in Immature Samples		
Proximal Metaphyseal Microscopic Cracking	The cortex of the proximal metaphyseal surface contains at least one crack that is visible at 10x magnification				

Proximal Metaphyseal Marginal Loss	There is localized loss of the proximal metaphyseal surface involving the perimeter				
Proximal Metaphyseal Central Loss	There is localized loss of the proximal metaphyseal surface that does not involve the perimeter				
Distal Metaphyseal Macroscopic Cracking	The cortex of the distal metaphyseal surface contains at least one crack that is visible to the naked eye				
Distal Metaphyseal Microscopic Cracking	The cortex of the distal metaphyseal surface contains at least one crack that is visible at 10x magnification				
Distal Metaphyseal Marginal Loss	There is localized loss of the distal metaphyseal surface involving the perimeter				
Distal Metaphyseal Central Loss	There is localized loss of the distal metaphyseal surface that does not involve the perimeter				

Following their exposure time, the radii were photographed and observed under a Leica stereomicroscope at 10X magnification. The microscopic traits were recorded under the microscope, while macroscopic traits were evaluated using the naked eye. All changes were recorded as present or absent in each bone specimen and then converted into a frequency of occurrence for each month. Any differences between the weathering patterns of the immature and mature bones, throughout the postmortem interval, were evaluated using a Kolmogorov Smirnov test, which compared the frequency distribution of each examined weathering variable between the immature and mature groups. Associations between the physical changes of the bone surface, and the compositional changes through time were also evaluated using a Kolmogorov Smirnov test, which compared the distribution of each weathering variable and weight loss on ignition component within the immature and mature groups.

3.5. Results

3.5.1. Weight Loss on Ignition Analysis

The weight loss on ignition protocol allowed successful approximation of the bone composition throughout the experimental interval. The similarity of the unbound water, bound water, collagen and mineral values between sampling months was tested using Kruskal Wallis and post-hoc comparisons. The results are given in the appendix (Appendix I-P), and they revealed several trends. The unbound water (Figure 7) underwent a statistically significant increase in the immature samples between months 0 and 8, then a decrease from month 9 to 12. In the mature bone samples, on the other hand, unbound water remained relatively constant until month 3, then experienced a statistically significant increase to a relative plateau after month 8.

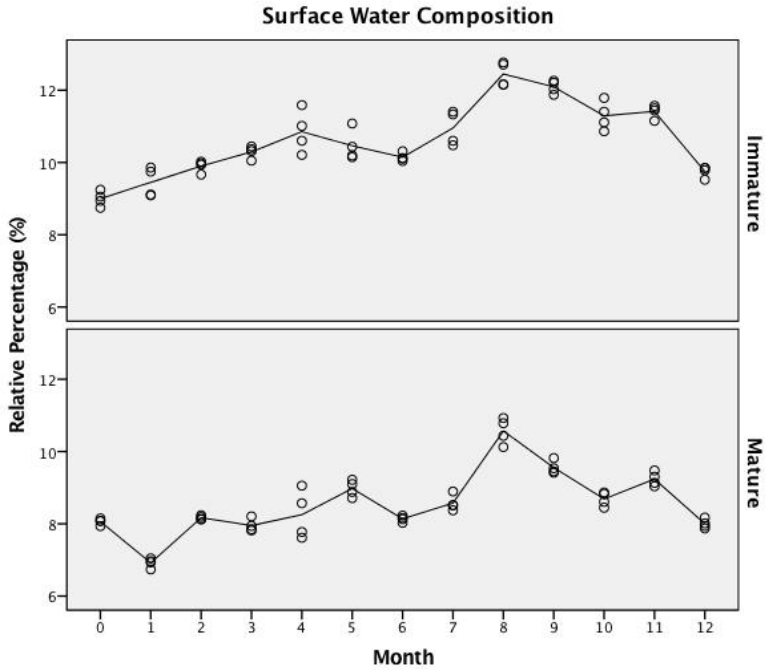


Figure 7: Changes in unbound water content (% of total weight) over the 12-month interval (n=104)

The bound water content of the immature bones (Figure 8) remained relatively constant until month 7, when it exhibited a statistically significant decrease to the final month of analysis. In the mature samples, the bound water content increased statistically significantly between months 2 to 7, then remained relatively constant for the remainder of the experimental interval.

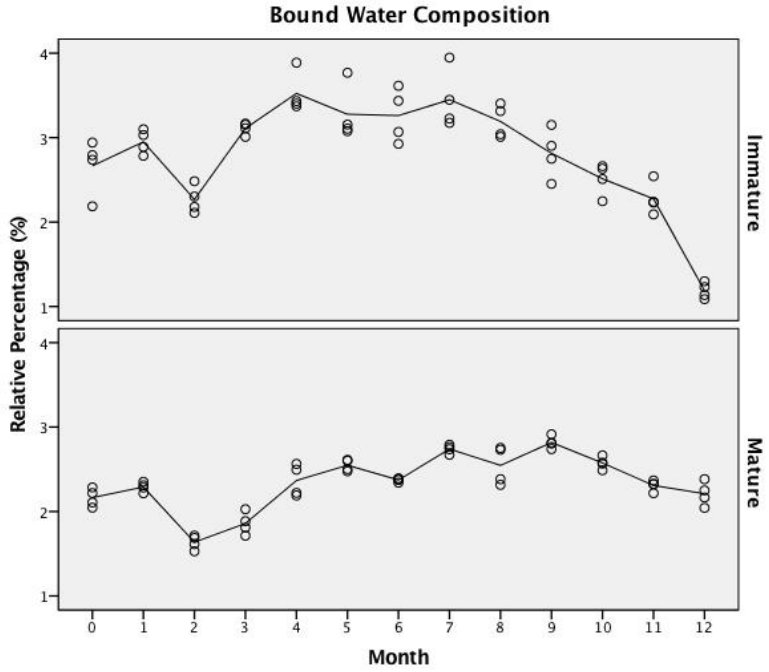


Figure 8: Changes in bound water content (% of total weight) over the 12-month interval (n=104)

The collagen content (Figure 9) decreased a statistically significant amount between months 0 to 4, then remained relatively constant until the final month of analysis in both the immature and mature bone samples. Despite the similar trend, the relative collagen decrease is much larger in the immature bone samples.

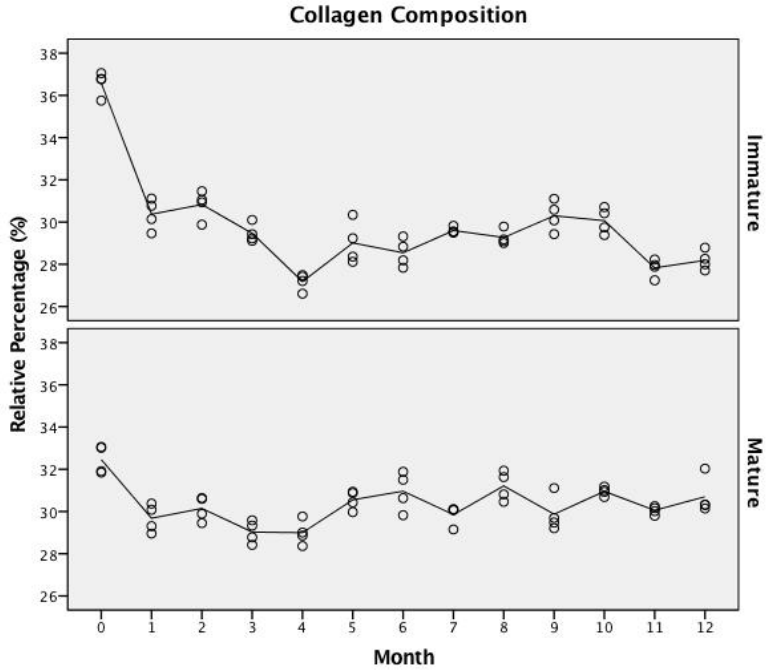


Figure 9: Changes in collagen content (% of total weight) over the 12-month interval (n=104)

Finally, the relative mineral content (Figure 10) showed a statistically significant increase in the immature samples until month 4, then again between months 8 and 12. In the mature samples, however, the mineral content increased insignificantly within the first month, then experienced a statistically significant decrease to month 8, after which it does not change significantly.

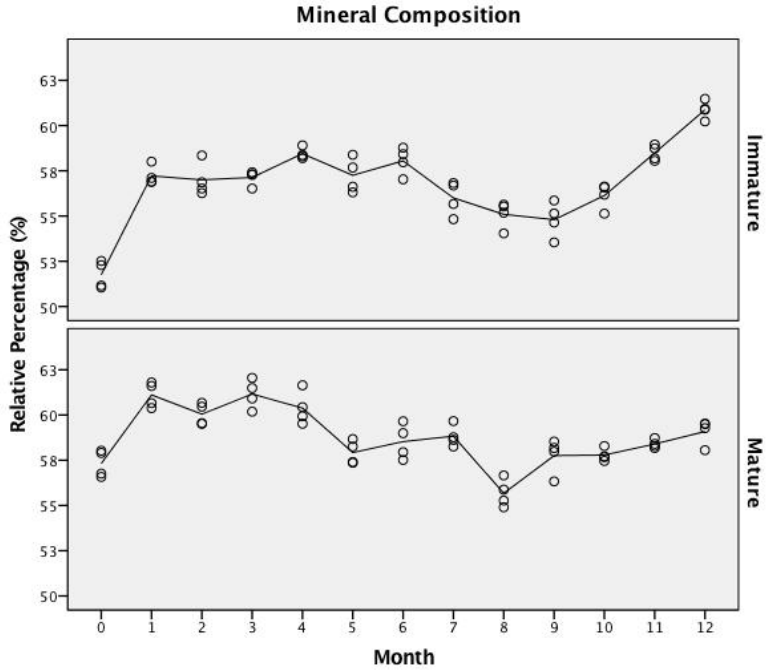


Figure 10: Changes in mineral content (% of total weight) over the 12 month interval (n=104)

The Mann-Whitney U results, reported in Table 8, show that the median values of unbound water, bound water, collagen, and mineral differed significantly between the two maturity groups. These differences were maintained throughout a majority of the experimental interval.

Table 8: Mann-Whitney U test results when comparing the medians of each weight loss on ignition variable between immature and mature groups (*Significant when p<0.05)

Month	*Unbound Water*	*Bound Water*	*Collagen*	*Mineral*
0	Z= -2.309 p= 0.029 *	Z= -2.309 p= 0.029 *	Z= -2.309 p= 0.029 *	Z= -2.309 p= 0.029 *
1	Z= -2.309 p= 0.029 *	Z= -2.309 p= 0.029 *	Z= -1.443 p= 0.200	Z= -2.309 p= 0.029 *
2	Z= -2.309 p= 0.029 *	Z= -2.309 p= 0.029 *	Z= -1.443 p= 0.200	Z= -2.309 p= 0.029 *
3	Z= -2.309 p= 0.029 *	Z= -2.309 p= 0.029 *	Z= -0.866 p= 0.486	Z= -2.309 p= 0.029 *
4	Z= -2.309 p= 0.029 *	Z= -2.309 p= 0.029 *	Z= -2.309 p= 0.029 *	Z= -2.309 p= 0.029 *
5	Z= -2.309 p= 0.029 *	Z= -2.309 p= 0.029 *	Z= -2.021 p= 0.057	Z= -0.866 p= 0.486
6	Z= -2.309 p= 0.029 *	Z= -2.309 p= 0.029 *	Z= -2.309 p= 0.029 *	Z= -0.577 p= 0.686
7	Z= -2.309 p= 0.029 *	Z= -2.309 p= 0.029 *	Z= -1.155 p= 0.343	Z= -2.309 p= 0.029 *
8	Z= -2.309 p= 0.029 *	Z= -2.309 p= 0.029 *	Z= -2.309 p= 0.029 *	Z= -0.866 p= 0.486
9	Z= -2.309 p= 0.029 *	Z= 0.000 p= 1.00	Z= -0.577 p= 0.686	Z= -2.309 p= 0.029 *
10	Z= -1.452 p= 0.200	Z= -2.309 p= 0.886	Z= -2.021 p= 0.057	Z= -2.309 p= 0.029 *
11	Z= -2.309 p= 0.029 *	Z= -0.577 p= 0.686	Z= -2.309 p= 0.029 *	Z= -0.289 p= 0.886
12	Z= -2.309 p= 0.029 *	Z= -2.309 p= 0.029 *	Z= -2.309 p= 0.029 *	Z= -2.309 p= 0.029 *

The Kolmogorov Smirnov tests comparing the distributions of the unbound water, bound water, collagen, and mineral values between the two maturity groups, through time are given in Table 9. These tests reveal that the distributions of the immature and mature bone components through time, are not the same for any of the weight loss on ignition variables. This is supported by the Mann-Whitney U and Kruskal Wallis results,

which indicate that there is a difference in both median values of each component, as well as their variation through time.

Table 9: Kolmogorov Smirnov test results when comparing the distributions of each weight loss on ignition variable between immature and mature groups. (*Significant when p<0.05)

Weight Loss on Ignition Variable	*D-Value*	*p- Value*
Unbound Water	3.922	0.000 *
Bound Water	2.844	0.000 *
Collagen	1.863	0.002 *
Mineral	2.451	0.000 *

3.5.2. Bone Surface Weathering Analysis

Observational data revealed that several physical bone changes were specific to a single maturity group: multidirectional cortical roughness, and proximal and distal cortical peeling were found only in the mature samples. Table 10 shows the results of the Kolmogorov Smirnov tests, where the frequency distributions of the remaining weathering variables were compared between the immature and mature groups over time. The comparisons through time indicate that there is a statistically significant difference between not only the distributions of multidirectional cortical roughness and cortical peeling, but also microscopic cracking of the metaphyseal surfaces, macroscopic cracking in the distal metaphyseal surface, and localized loss in the proximal metaphyseal surface. These results are consistent with the observational data. Microscopic cracking of the proximal and distal metaphyseal surfaces was found in both immature and mature groups after the first month, but at a higher frequency in the mature bone samples. Distal macroscopic cracking was found in only one immature sample when fresh, but several mature bone samples throughout the entire experimental interval. Finally, localized loss of the proximal metaphyseal surface, both marginally and centrally, was found predominantly in the immature bone samples after the first month of breakdown. There was no significant difference between the other quantified variables: distal metaphyseal surface loss, which was observed in samples from both maturity groups, macroscopic cracking of the proximal metaphyseal surface, which was found in

very few samples, and linear cortical roughness, which was observed extensively in both immature and mature samples, beginning in month 3 in the mature bones, and month 4 in the immature bones.

Table 10: Kolmogorov Smirnov test results when comparing the immature and the mature distributions of each weathering variable over time (* highlights significant values at p<0.05).

Weathering Variable	*D-Value*	*p- Value*
Linear Cortical Roughness	0.588	0.879
Multidirectional Cortical Roughness	1.373	0.046 *
Proximal Cortical Peeling	2.353	0.000 *
Distal Cortical Peeling	1.961	0.001 *
Proximal Metaphyseal Macroscopic Cracking	0.784	0.570
Proximal Metaphyseal Microscopic Cracking	1.961	0.001 *
Proximal Metaphyseal Marginal Loss	1.569	0.015 *
Proximal Metaphyseal Central Loss	1.569	0.015 *
Distal Metaphyseal Macroscopic Cracking	2.157	0.000 *
Distal Metaphyseal Microscopic Cracking	1.569	0.015 *
Distal Metaphyseal Marginal Loss	0.392	0.998
Distal Metaphyseal Central Loss	0.588	0.879

When comparing the compositional changes in the bone material with the physical changes, Kolmogorov Smirnov tests showed that in both the immature (Table 11) and mature bone samples (Table 12), the distribution of all weight loss on ignition variables differed in a statistically significant manner from the distribution of the weathering variables. This indicates that physical bone breakdown occurred independently of the compositional changes that took place.

Table 11: Kolmogorov Smirnov test results when comparing the distributions of each weight loss on ignition variable to the measured weathering variables in the immature bone sample. (*highlights distributions that <u>do not</u> differ significantly at p>0.05)

Weathering Variable	*Unbound Water*	*Bound Water*	*Collagen*	*Mineral*
Linear Cortical Roughness	D= 0.615 p= 0.015	D= 0.615 p= 0.015	D= 0.539 p= 0.046	D= 0.693 p= 0.004
Multidirectional Cortical Roughness	D= 1.000 p= 0.000	D= 1.000 p= 0.000	D= 1.000 p= 0.000	D= 1.000 p= 0.000
Proximal Cortical Peeling	D= 1.000 p= 0.000	D= 1.000 p= 0.000	D= 1.000 p= 0.000	D= 1.000 p= 0.000
Distal Cortical Peeling	D= 0.923 p= 0.000	D= 0.923 p= 0.000	D= 1.000 p= 0.000	D= 1.000 p= 0.000
Proximal Metaphyseal Macroscopic Cracking	D= 1.000 p= 0.000	D= 1.000 p= 0.000	D= 1.000 p= 0.000	D= 1.000 p= 0.000
Proximal Metaphyseal Microscopic Cracking	D= 0.769 p= 0.001	D= 0.769 p= 0.001	D= 0.615 p= 0.015	D= 0.693 p= 0.004
Proximal Metaphyseal Marginal Loss	D= 1.000 p= 0.000	D= 1.000 p= 0.000	D= 0.923 p= 0.000	D= 0.923 p= 0.000
Proximal Metaphyseal Central Loss	D= 0.846 p= 0.000	D= 0.846 p= 0.000	D= 0.846 p= 0.000	D= 0.615 p= 0.015
Distal Metaphyseal Macroscopic Cracking	D= 0.923 p= 0.000	D= 0.923 p= 0.000	D= 1.000 p= 0.000	D= 1.000 p= 0.000
Distal Metaphyseal Microscopic Cracking	D= 0.769 p= 0.001	D= 0.769 p= 0.001	D= 0.615 p= 0.015	D= 0.539 p= 0.046
Distal Metaphyseal Marginal Loss	D= 1.000 p= 0.000	D= 1.000 p= 0.000	D= 0.923 p= 0.000	D= 0.923 p= 0.000
Distal Metaphyseal Central Loss	D= 1.000 p= 0.000	D= 1.000 p= 0.000	D= 0.923 p= 0.000	D= 0.539 p= 0.046

Table 12: Kolmogorov Smirnov test results when comparing the distributions of each weight loss on ignition variable to the measured weathering variables in the mature bone sample. (*highlights distributions that <u>do not</u> differ significantly at p>0.05)

Weathering Variable	*Unbound Water*	*Bound Water*	*Collagen*	*Mineral*
Linear Cortical Roughness	D= 0.692 p= 0.004	D= 0.692 p= 0.004	D= 0.692 p= 0.004	D= 0.539 p= 0.046
Multidirectional Cortical Roughness	D= 0.539 p= 0.046	D= 0.539 p= 0.046	D= 0.615 p= 0.015	D= 0.615 p= 0.015
Proximal Cortical Peeling	D= 0.539 p= 0.046	D= 0.539 p= 0.046	D= 0.769 p= 0.001	D= 0.846 p= 0.000
Distal Cortical Peeling	D= 0.539 p= 0.046	D= 0.539 p= 0.046	D= 0.846 p= 0.000	D= 1.000 p= 0.000
Proximal Metaphyseal Macroscopic Cracking	D= 0.692 p= 0.004	D= 0.692 p= 0.004	D= 0.923 p= 0.000	D= 1.000 p= 0.000
Proximal Metaphyseal Microscopic Cracking	D= 1.000 p= 0.000	D= 1.000 p= 0.000	D= 0.923 p= 0.000	D= 0.923 p= 0.000
Proximal Metaphyseal Marginal Loss	D= 0.923 p= 0.000	D= 0.923 p= 0.000	D= 0.769 p= 0.001	D= 0.539 p= 0.046
Proximal Metaphyseal Central Loss	D= 0.539 p= 0.046	D= 0.539 p= 0.046	D= 0.769 p= 0.001	D= 0.923 p= 0.000
Distal Metaphyseal Macroscopic Cracking	D= 0.846 p= 0.000	D= 0.846 p= 0.000	D= 0.846 p= 0.000	D= 0.539 p= 0.046
Distal Metaphyseal Microscopic Cracking	D= 0.923 p= 0.000	D= 0.923 p= 0.000	D= 0.923 p= 0.000	D= 0.923 p= 0.000
Distal Metaphyseal Marginal Loss	D= 0.846 p= 0.000	D= 0.846 p= 0.000	D= 0.846 p= 0.000	D= 0.769 p= 0.001
Distal Metaphyseal Central Loss	D= 0.846 p= 0.000	D= 0.846 p= 0.000	D= 0.769 p= 0.001	D= 0.539 p= 0.046

3.6. Discussion

3.6.1. Weight Loss on Ignition

The results of this study are consistent with the current literature pertaining to bone composition; the immature bones contained a higher water and collagen content, and a lower mineral content than the mature bones. The normative values of mature human bone composition are known to be approximately 20-25% organic collagen (Buckberry 2000, Hedges and Millard 1995b, Nielsen-Marsh and Hedges 2000) and 60-70% mineral (Boskey 2014), with the remaining attributed to water. A study by Chittenden et al. (2015) analyzed fresh porcine femur bone of varying maturities, and determined that the relative collagen content of porcine bone is close to 30% in pigs aged 1 month (the age of our immature samples) but decreases significantly by 6 months of age (the age of our mature samples) and that the mineral content increases from approximately 35% to 60% during this time. The results from our study, while inconsistent with the values for human bone, are somewhat similar to the porcine values obtained by Chittenden et al. (2015). The relative percent composition of collagen was determined to be approximately 39% in the immature bone, and 35% in the mature bone. The relative percent composition of mineral was approximately 47% in the immature samples, and 54% in the mature samples. These results further support the body of evidence for the dependence of bone composition on maturity.

The compositional changes that are known to occur in response to the maturity level of bone are related to the overall structural changes, and affect the way in which the bone is able to interact with its environment. Immature bone is less mineralized than mature bone, with the hydroxide and carbonate ions arranged into a lattice of small crystals (Guy 1997). This arrangement facilitates the extensive collagen matrix, and elastic and porous bone structure (Bello and Andrews 2006, Buckberry 2000, Djuric et al. 2011, Gordon and Buikstra 1981, Guy 1997, Lewis 2007, Manifold 2010, 2012, 2013, Mays 2010, Walker et al. 1988). This high level of porosity creates a large total surface area, over which environmental assault from agents such as microorganisms and ground water can occur (Boaks et al. 2014, Buckberry 2000, Djuric et al. 2011, Garland 1987, Manifold 2012, Mays 2010). A mature bone, on the other hand, incorporates ions from the environment into the mineral structure and increases the size of its crystals into a more stable formation, reducing the free energy of the structure (Guy 1997, Hedges

and Millard 1995b, Mays 2010). These changes result in a bone with a higher mineral content, less collagen elasticity, and decreased porosity, that consequently has less bone surface exposed to the environment (Guy 1997). The expected variation in composition and porosity due to maturity likely played a role in determining the compositional changes that were observed within each maturity group.

The changes in relative unbound water, bound water, collagen, and mineral content (Figure 7-10) that were observed in this study differed significantly between the immature and mature bone samples, as predicted. The most interesting changes occurred within the first month, and final 4 months of observation (Table 8). In the first month of being buried, the relative collagen content decreased, and relative mineral content increased in both the immature and mature bone samples. This can be interpreted as a loss of collagen due to infiltration of the bone material by ground water and bacteria. Ground water, the moisture within the soil, is the medium through which recrystallization, dissolution, hydrolysis, ion exchange, and microbial attack all affect bone material (Hedges and Millard 1995a, Manifold 2012, 2013, Mays 2010, Nielsen-Marsh and Hedges 2000). It is considered to be the most influential agent in bone breakdown (Manifold 2012, Mays 2010), and can cause mineral dissolution due to ion imbalances with the environment, as well as rapid collagen hydrolysis (Garland 1987, Manifold 2012, 2013, Mays 2010, Nielsen-Marsh and Hedges 2000), which may have happened within the first month of this study. Bacteria, on the other hand, consume bone collagen by dissolving the linking hydroxyapatite mineral and then redepositing it as they move through the bone material (Jans et al. 2004, Mays 2010). While this may have affected the composition of both the immature and mature bone samples in this study, the relative initial changes in the immature bones were much greater; this may be explained by the maturity-dependent changes in the porosity of bone. In a porous immature structure, ground water and bacteria are able to move through the naturally occurring spaces within the bone more easily, allowing the breakdown of its structure to be much more rapid (Jans et al. 2004).

In the final 4 months, the immature samples experienced a relative increase in mineral content and a relative decrease in the bound water content. The mature bone samples, on the other hand, did not exhibit any changes in the final months of experimentation. These differences may also be explained by the maturity-dependent differences in bone structure. The high porosity of the immature bone would have been

further increased by the collagen hydrolysis that had already occurred in the first month of degradation, creating an even larger area over which environmental attack could occur. The increase in relative mineral content could have occurred as a product of environmental infiltrations, which are exchanges with the soil to include larger ions into the mineral matrix, or inclusions, which occur when minerals enter the bone pores and then precipitate into solid materials such as calcite, pyrite, or quartz (Garland 1987, Hedges and Millard 1995b). These processes could have displaced bound water molecules, resulting in the observed loss of relative bound water content.

The compositional analyses performed in this study suggest that within the early postmortem period in a buried degradation environment, the relative composition of bone and its changes through time vary according to the maturity of the skeletal material. The significant changes in relative collagen and mineral content that occurred in the immature samples, during the first and final 4 months of the experimental interval, suggest that immature bone is more susceptible to compositional breakdown than that of mature bone material.

3.6.2. Bone Surface Weathering

Similar to the compositional changes, the bone weathering analysis indicates that physical changes of bone material, relative to environment, also vary according to the maturity of the bone. The outer cortex of the mature bone samples demonstrated multidirectional cortical roughness, and cortical peeling throughout the entirety of the shaft, while these changes were not observed in the immature samples. Further, the presence of metaphyseal surface microscopic cracking and distal metaphyseal surface macroscopic cracking were statistically more frequent in the mature bone samples. The immature samples were only found to have a higher frequency of cortical bone loss in the proximal metaphyseal surface than the mature bone counterparts. This indicates that the mature bone samples may have been more susceptible to physical breakdown than the immature bones; this is contrary to popular expectation.

The differences observed in the types and frequencies of physical weathering that affected the immature and mature bone samples can be explained by developmental differences in the structure of the bone material. Bone begins as a cartilaginous precursor, which gets replaced by osseous tissue through a process known

as endochondral ossification (Hillier and Bell 2007, Manifold 2014). This tissue is laid down in concentric rings as osteon structures form around the Haversian canals that provide a route for bone vasculature (Hillier and Bell 2007, Manifold 2008). During the initial stages of ossification, the osteons are arranged longitudinally, radially, and circumferentially; this structure is classified as plexiform bone (Hillier and Bell 2007). With maturity, however, some areas of plexiform bone are replaced by a lamellar structure (Manilay et al. 2013), in which all the Haversian systems and accompanying concentric bone layers run longitudinally through the bone (Hillier and Bell 2007). This mature lamellar bone becomes increasingly resistant to compressive forces, however, the new parallel structure, composed of more mineral and less collagen, is much less elastic (Guy 1997). The immature and mature bones used in this study contain varying quantities of plexiform bone, with the more mature bone containing less (Hillier and Bell 2007, Manilay et al. 2013); this structural difference played a determining role in the interactions between the environment and bone material.

The samples in this study exhibited physical changes which vary according to the maturity of the bone, and that can be explained by the structure of its material. The immature bones were affected by fewer types of physical changes; however, they were more frequently observed to have localized loss in the cortex of their metaphyseal surfaces. The loss documented in this region can be explained by its fragile nature (Djuric et al. 2011, Lewis 2007). Resorption and creation of bone material is constantly occurring in the metaphyseal growth plate (Clarke 2008), which leaves it thin and easily degraded by external forces such as bacteria (Jans et al. 2004, Mays 2010), or groundwater (Djuric et al. 2011, Lewis 2007). The bacteria and ground water present in this study may have played a role in the observed loss of collagen through bacterial consumption (Jans et al. 2004, Mays 2010) or hydrolysis by water (Garland 1987, Manifold 2012, 2013, Mays 2010, Nielsen-Marsh and Hedges 2000), which would have left a brittle mineral scaffold that was susceptible to breakage (Dupras and Schultz 2012, Junod and Pokines 2012). These processes would have also occurred in the mature bone samples, which were found to have a higher frequency of cortical roughness and peeling, as well as cracking in the metaphyseal surfaces. While the cracking of the metaphyseal surfaces is more easily explained by the loss of collagen content to both bacterial degradation and ground water infiltration, the difference in cortical peeling and roughness is perhaps best explained by the varying amounts of plexiform bone in the

samples. With a higher degree of lamellar structure, the outer concentric layers of bone could be more easily separated from one another; the multidirectionality and elastic qualities of a less mature bone would prevent this from occurring.

The differences observed in the physical changes of immature and mature bone, within a buried environment, suggest that bone maturity plays a major role in determining the type of breakdown that occurs in the early postmortem interval. A significantly higher frequency of several types of weathering changes was observed in the mature samples, beginning as early as the first month of degradation, suggesting that they are more susceptible to the physical assault of their environment than less mature bone.

3.6.3. The Role of Compositional Change in Physical Destruction

The combination of weight loss on ignition results and the bone weathering results suggests that, within the early postmortem interval of buried bone degradation, maturity plays an important role in the type of changes observed. Mature bone was found to be more susceptible to physical destruction by its environment, while immature bone underwent more pronounced compositional changes. Despite these differences, the changes in composition of both the immature and mature bones were not found to be associated with the physical weathering of the bones.

3.6.4. Limitations of this Study

This study provides a controlled comparison of the compositional and physical breakdown of immature and mature bone material. While the results are valuable to informing the archaeological community as to the relative survival of immature and mature bone material, it does not attempt to create a scale with which the timeframe of breakdown can be estimated. This stems from the initial maceration protocol of the experiment. Mechanically removing the flesh from the bones eliminated the medium through which autolysis and microbial attack normally stem (Bilheux et al. 2015, Donaldson and Lamont 1979, Ross and Cunningham 2011). It was, however, necessary for examining maturity as the causative factor in differential bone decay, as it allowed all external conditions to be held constant across all samples. This maceration protocol,

along with the setup, weight loss on ignition, and bone weathering protocols, provides the limiting factors in this study.

The experimental setup was carried out in a monitored greenhouse environment to ensure that all bone samples were exposed to equivalent external conditions at any given time. The soil environment, however, may have played a role in the types of weathering observed, and despite the ability to equilibrate the depositional environment across all experimental plots, it was not possible to hold it constant through time. The soil was of a neutral pH, with a high organic content, and a high drainage potential. A neutral pH is preservative, potentially slowing any processes that would have been visible in a harsher environment. The organic component and drainage of the soil had the ability to leach mineral content from the bone material, however, the water flow was kept at a minimum to prevent this. As a result, the potential bone changes may have been dampened. The variation in temperature and humidity through time may have also played a role. A non-linear accumulation of degradative affects, containing plateaus or sharp inclines, would have prevented normalization of the breakdown rate per unit time. This was, however, mediated by the greenhouse environment, which allowed prevention of large fluctuations in temperature and moisture throughout the experimental interval.

The weight loss on ignition analysis necessitated the destruction of a section from each sample; this, along with the removal of each bone sample from their burial environment, increased the number of bone samples needed. Requiring a large number of bones to be mechanically defleshed dictated the number of samples that could be prepared, which in turn caused the problem of sample size. With only 4 samples per maturity group, any individual variation or outliers could have caused extreme fluctuations in compositional values through time. Further, because a new set of bones were required for each month of analysis, the compositional and physical analyses could not be repeated on the same bones throughout the year-long study interval. This also contributed to the potential problem caused by individual variation. This was, however, addressed by using the median values of the samples for each month. Finally, the furnace used for this weight loss on ignition protocol was often inaccurate when setting temperatures below 100°C. Fluctuations in the temperature of the furnace may have influenced the distinction between unbound and bound water by including bound water in the unbound water measurement.

The bone weathering protocol was designed to quantify only the changes that were observed in the bone samples. Only cortical roughness and metaphyseal surface cortical bone loss have been documented in any previous short-term taphonomy studies, such as those performed by Cunningham et al. (2011) and Janjua and Rogers (2008), while the other types of weathering have not been noted by any previous studies. Further, they are on a much smaller scale than the well-known stages laid out by Behrensmeyer in 1978, which looks at gross bone destruction instead of minute changes. Because of this, the observations are not well standardized against any previous literature; this has been mediated by including a description and visual representation of each variable for future use in short-term taphonomy studies.

Despite the limitations imposed by the protocols used in this study, the methods were extremely cost and time effective, and well-suited for the necessary analyses. Careful consideration of the equipment, samples, and experimental time frame allowed for the problems to be addressed and minimized as much as possible.

3.6.5. Significance

This project provides a novel comparison of the compositional and physical destruction of immature and mature bone material in a buried environment. Previous taphonomy studies have attempted to quantify changes within adult bone, and the effects of these changes on the survival of bone material in the archaeological record. These studies, including those by Behrensmeyer (1978) and Andrews (1995), look at the long-term survival of mature bone material and document extensive bone cracking and fragmentation, the likes of which have not been observed in any short-term studies. This poses problems for the interpretation of immature bone weathering, as any previous studies (Cunningham et al. 2011, Rogers and Janjua 2008) documented their changes only within the early postmortem interval, or did not do so in a comparative nature to mature bone. Therefore, it is not known how the differences observed in the early stages of bone weathering will affect the long-term survival of immature archaeological bone.

The results of this study help to inform the archaeological community by providing evidence for the significant role that maturity plays in determining the type of bone breakdown observed in bone material. While immature bones were found to be most heavily impacted by compositional changes, they were impacted by fewer types of

physical weathering than their mature bone counterparts. This does not support, nor refute, the notion that taphonomy can be a significant causative agent in limiting the number of immature skeletal remains identified in an archaeological excavation. It does, however, indicate that further research is needed to identify how these maturity-dependent differences in early bone breakdown will affect the long-term survival of the bones. Further, it indicates that archaeological, ecological, and paleoecological taphonomic interpretations of weathered immature bone material, when performed using rates based on the changes of mature bone, should be done so with caution. If maturity-dependent intrinsic bone qualities are associated with differential bone weathering over an extended postmortem interval, then the accuracy of current weathering indices will be compromised.

3.7. Conclusion

Within the early postmortem interval of a buried environment, the compositional and physical changes that occur in bone material vary according to the maturity of the bone. This is consistent with not only the theory that maturity-dependent intrinsic bone qualities should affect their weathering, but also with the preliminary results of Gonzales et al (2011), which stated that bone of varying maturities will degrade differently. The present study suggests that immature bone changes primarily in composition, and is affected by weathering later on, whereas mature bone experiences physical changes early, and the composition is largely unchanged within the first year. Further, the compositional changes were observed to be unrelated to any specific physical weathering of the bones. These results indicate that maturity mediates the way in which a bone degrades, but it cannot address the speed to which this bone destruction will occur. Further research into defining how the initial maturity-dependent differences in breakdown translate into the longer-term survival of immature bone material is integral to understanding the recovery patterns of archaeological assemblages and, subsequently, the demography and health of past populations.

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Chapter 4.

The Effect of Differing Depositional Conditions

Within the early postmortem period, the compositional and surface alterations that occur in skeletal material appear to be influenced by the maturity of the bone. This study presented evidence for the association between weathering and maturity within both a subaerial and buried environment. In both postmortem locations, the immature bone material was found to undergo more pronounced compositional changes, with physical surface breakdown occurring later, and to a lesser degree than the mature bone samples. The mature bones did not vary significantly in their composition through time, but they were found to be more susceptible to physical breakdown by their environment. Finally, weathering traits such as cortical peeling in the bone shaft were only observed in mature samples. These results build off of the preliminary observations of Gonzales et al. (2011), who determined that the age of guanaco bone determined its degree of weathering in a subaerial environment, within the first four years postmortem. Their study did not, however, assess this differential breakdown within varying environments.

The degradation of bone material is heavily influenced by its local environment, with factors such as soil composition, exposure to the elements and bacteria, and slight temperature fluctuations affecting the physical state of the bone (Behrensmeyer 1978, Buckberry 2000, Cunningham et al. 2011, Ubelaker 1997). Previous studies have determined that open environments with a high degree of exposure to scavengers and environmental assault will cause quick degradation, while stable environments with limited fluctuations in temperature and moisture, and little disturbance will tend to preserve bone material (Junod and Pokines 2012, Madgewick and Mulville 2012). The current study also found that environment was found to mediate the extent to which maturity affected bone weathering, but the subaerial environment was found to be more protective of the bone samples than that of the buried environment. In the subaerial samples, there were no major changes in the mature bone composition, with the immature samples exhibiting their major increase in relative mineral, and decrease in relative collagen within the final 3 months of the experimental interval. Further, there was no recorded roughness in the shaft cortex, but peeling of this region was abundant in the mature bone samples. In the buried environment, on the other hand, the compositional

changes occurred rapidly within the first month of burial; the samples from both maturity groups experienced a relative decrease in collagen and increase in mineral content. As observed in the subaerial environment, there was also a major compositional change of the immature bone samples within the final months of the experiment, but these samples underwent a relative decrease in bound water when their relative mineral content increased. The physical weathering of the buried samples was also different; the integrity of the cortical bone structure was compromised in the shaft of samples from both maturity groups. The mature bones exhibited linear, and multidirectional cortical roughness, while the immature samples demonstrated only linear cortical roughness. Peeling, however, was much less frequent in this buried environment.

The differences observed between the two experimental locations can be interpreted with the environmental variables being a causative factor, but the unusually more rapid decay in the buried samples is most likely due to the removal of flesh. The subaerial samples were, most likely, only affected by sunlight-induced changes, and bacterial infiltration, as groundwater and soil were not involved factors. Ultraviolet radiation would likely have caused gradual collagen degradation, while small sunlight induced temperature fluctuations could have caused shrinkage of the cortex to result in peeling (Dupras and Schultz 2012, Zayat et al. 2007). Bacterial infiltration would have caused collagen breakdown in both environments (Jans 2004, Mays 2010). The presence of groundwater in the buried environment could have allowed rapid collagen hydrolysis within the first month of the experiment (Garland 1987, Manifold 2012, 2013, Mays 2010, Nielsen-Marsh and Hedges 2000). The soil in this location also facilitated infiltrations, inclusions, and ion exchanges with the bone material (Hedges and Millard 1995, Manifold 2012, 2013, Mays 2010, Nielsen-Marsh and Hedges 2000), which could have contributed to the relative mineral increase, without a corresponding change in collagen, observed in these immature samples. Further, the more rapid breakdown in the buried environment may be attributed to the lack of disturbance, scavenging, and large temperature fluctuations in this highly controlled experiment. Animals were not present; however, they often scatter, gnaw, partially digest, or fragment bones when they are left to decay without burial (Andrews 1995, Byers 2011, Haglund 1997). Large temperature fluctuations were also prevented in this study, although some expansion and contraction of the bones was still possible and this would have compromised the

structural integrity and allowed the infiltration of groundwater in the buried environment (Byers 2011, Junod 2012, Manifold 2012).

The results of this study support the previous literature, which states that environment plays a determining role in the breakdown of bone material. Further, this study was able to determine that environment will mediate the extent to which maturity induces differential bone breakdown. While a buried environment will prompt initial changes in bone chemistry, the same pattern of comparative weathering occurs between the two maturity groups in both environments. This suggests that within the first year in any equivalent environments, an immature bone will undergo more pronounced compositional changes than their mature bone counterparts, while the mature bones will exhibit a greater physical reaction to the environment. How this initial difference translates into the longer-term survival of the bone material, however, requires future study of a longer experimental interval.

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Appendix A.

Kruskal Wallis post-hoc pairwise comparison with Bonferonni correction for the unbound water in subaerial immature bone material. Test statistic (TS) and p-values given for the comparison between each month.

Month	*0*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
0	-	TS= 1.073 p= 1.000	TS= -2.065 p= 1.000	TS= -1.120 p= 1.000	TS= -1.703 p= 1.000	TS= -0.933 p= 1.000	TS= 1.330 p= 1.000	TS= 1.003 p=1.000	TS= -2.216 p= 1.000	TS= -0.898 p= 1.000	TS= -0.863 p= 1.000	TS= 0.513 p= 1.000	TS= 1.936 p= 1.000
1		-	TS= -3.138 p= 0.133	TS= -2.193 p= 1.000	TS= -2.776 p= 0.429	TS= -2.006 p= 1.000	TS= 0.257 p= 1.000	TS= -0.070 p= 1.000	TS= -3.290 p=0.078	TS= -1.971 p= 1.000	TS= -1.936 p= 1.000	TS= -0.560 p= 1.000	TS= 0.863 p= 1.000
2			-	TS= 0.945 p= 1.000	TS= 0.362 p= 1.000	TS= 1.132 p= 1.000	TS= 3.395 p= 0.054	TS= 3.068 p= 0.168	TS= -0.152 p= 1.000	TS= 1.167 p= 1.000	TS= 1.202 p= 1.000	TS= 2.578 p= 0.775	TS= 4.001 p= 0.005
3				-	TS= -0.583 p= 1.000	TS= 0.187 p= 1.000	TS= 2.450 p= 1.000	TS= 2.123 p= 1.000	TS= -1.097 p= 1.000	TS= 0.222 p= 1.000	TS= 0.257 p= 1.000	TS= 1.633 p= 1.000	TS= 3.056 p= 0.175
4					-	TS= 0.770 p= 1.000	TS= 3.033 p= 0.189	TS= 2.706 p= 0.531	TS= -0.513 p= 1.000	TS= 0.805 p= 1.000	TS= 0.840 p= 1.000	TS= 2.216 p= 1.000	TS= 3.640 p= 0.021
5						-	TS= 2.263 p= 1.000	TS= 1.936 p= 1.000	TS= -1.283 p= 1.000	TS= 0.035 p= 1.000	TS= 0.070 p= 1.000	TS= 1.446 p= 1.000	TS= 2.870 p= 0.321
6							-	TS= -0.327 p= 1.000	TS= -3.546 p= 0.030	TS= -2.228 p= 1.000	TS= -2.193 p= 1.000	TS= -0.817 p= 1.000	TS=0.607 p= 1.000
7								-	TS= -3.220 p= 0.100	TS= -1.901 p= 1.000	TS= -1.866 p= 1.000	TS= -0.490 p= 1.000	TS= 0.933 p= 1.000
8									-	TS= 1.318 p= 1.000	TS= 1.353 p= 1.000	TS= 2.730 p= 0.494	TS= 4.153 p= 0.003
9										-	TS= 0.035 p= 1.000	TS= 1.411 p= 1.000	TS= 2.835 p= 0.385
10											-	TS= 1.377 p= 1.000	TS= 2.800 p= 0.399
11												-	TS= 1.423 p= 1.000
12													-

Appendix B.

Kruskal Wallis post-hoc pairwise comparison with Bonferonni correction for the bound water in subaerial immature bone material. Test statistic (TS) and p-values given for the comparison between each month.

Month	*0*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
0	-	TS= -0.723 p= 1.000	TS= 0.957 p= 1.000	TS= 0.327 p= 1.000	TS= -1.878 p= 1.000	TS= -1.003 p= 1.000	TS= -2.065 p= 1.000	TS= -2.356 p=1.000	TS= -1.283 p= 1.000	TS= 0.210 p= 1.000	TS= -2.753 p= 0.461	TS= -2.566 p= 0.802	TS= 1.306 p= 1.000
1		-	TS= 1.680 p= 1.000	TS= 1.050 p= 1.000	TS= -1.155 p= 0.429	TS= -0.280 p= 1.000	TS= -1.341 p= 1.000	TS= -1.633 p= 1.000	TS= -0.560 p=0.078	TS= 0.933 p= 1.000	TS= -2.030 p= 1.000	TS= -1.843 p= 1.000	TS= 2.030 p= 1.000
2			-	TS= -0.630 p= 1.000	TS= -2.835 p= 0.358	TS= -1.960 p= 1.000	TS= -3.021 p= 0.196	TS= -3.313 p= 0.072	TS= -2.240 p= 1.000	TS= -0.747 p= 1.000	TS= -3.709 p= 0.016	TS= -3.523 p= 0.033	TS= 0.980 p= 1.000
3				-	TS= -2.205 p= 1.000	TS= -1.330 p= 1.000	TS= -2.391 p= 1.000	TS= -2.683 p= 0.569	TS= -1.610 p= 1.000	TS= -0.117 p= 1.000	TS= -3.080 p= 0.162	TS= -2.893 p= 0.298	TS= 3.056 p= 1.000
4					-	TS= 0.875 p= 1.000	TS= -0.187 p= 1.000	TS= -0.478 p= 1.000	TS= 0.595 p= 1.000	TS= 2.088 p= 1.000	TS= -0.875 p= 1.000	TS= -0.688 p= 1.000	TS= 3.185 p= 0.113
5						-	TS= -1.062 p= 1.000	TS= -1.353 p= 1.000	TS= -0.280 p= 1.000	TS= 1.213 p= 1.000	TS= -1.750 p= 1.000	TS= -1.563 p= 1.000	TS= 2.310 p= 1.000
6							-	TS= -0.292 p= 1.000	TS= 0.782 p= 0.030	TS= 2.275 p= 1.000	TS= -0.688 p= 1.000	TS= -0.502 p= 1.000	TS=3.371 p= 0.058
7								-	TS= 1.073 p= 1.000	TS= 2.566 p= 0.802	TS= -0.397 p= 1.000	TS= -0.210 p= 1.000	TS= 3.663 p= 0.019
8									-	TS= 1.493 p= 1.000	TS= -1.470 p= 1.000	TS= -1.283 p= 0.494	TS= 2.590 p= 0.749
9										-	TS= -2.963 p= 0.238	TS= -2.776 p= 0.429	TS= 1.097 p= 1.000
10											-	TS= 0.187 p= 1.000	TS= 4.059 p= 0.004
11												-	TS= 3.873 p= 0.008
12													-

Appendix C.

Kruskal Wallis post-hoc pairwise comparison with Bonferonni correction for the collagen in subaerial immature bone material. Test statistic (TS) and p-values given for the comparison between each month.

Month	*0*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
0	-	TS= -3.126 p= 0.138	TS= -2.286 p= 1.000	TS= -2.240 p= 1.000	TS= -0.047 p= 1.000	TS= -1.750 p= 1.000	TS= -1.213 p= 1.000	TS= -1.423 p= 1.000	TS= -1.983 p= 1.000	TS= -3.523 p= 0.033	TS= -2.216 p= 1.000	TS= 0.677 p= 1.000	TS= -0.280 p= 1.000
1		-	TS= 0.840 p= 1.000	TS= 0.887 p= 1.000	TS= 3.079 p= 0.162	TS= 1.376 p= 1.000	TS= 1.913 p= 1.000	TS= 1.703 p= 1.000	TS= 1.143 p= 1.000	TS= -0.397 p= 1.000	TS= 0.910 p= 1.000	TS= 3.803 p= 0.011	TS= 2.846 p= 0.345
2			-	TS= 0.047 p= 1.000	TS= 2.240 p= 1.000	TS= 0.537 p= 1.000	TS= 1.073 p= 1.000	TS= 0.863 p= 1.000	TS= 0.303 p= 1.000	TS= -1.236 p= 1.000	TS= 0.070 p= 1.000	TS= 2.963 p= 0.238	TS= 2.006 p= 1.000
3				-	TS= 2.193 p= 1.000	TS= 0.490 p= 1.000	TS= 1.026 p= 1.000	TS= 0.817 p= 1.000	TS= 0.257 p= 1.000	TS= -1.283 p= 1.000	TS= 0.023 p= 1.000	TS= 2.916 p= 0.276	TS= 1.960 p= 1.000
4					-	TS= -1.703 p= 1.000	TS= -1.166 p= 1.000	TS= -1.376 p= 1.000	TS= -1.936 p= 1.000	TS= -3.476 p= 0.040	TS= -2.170 p= 1.000	TS= 0.723 p= 1.000	TS= -0.233 p= 1.000
5						-	TS= 0.537 p= 1.000	TS= 0.327 p= 1.000	TS= -0.233 p= 1.000	TS= -1.773 p= 1.000	TS= -0.467 p= 1.000	TS= 2.426 p= 1.000	TS= 1.470 p= 1.000
6							-	TS= -0.210 p= 1.000	TS= -0.770 p= 1.000	TS= -2.310 p= 1.000	TS=-1.003 p= 1.000	TS= 1.890 p= 1.000	TS= 0.933 p= 1.000
7								-	TS= -0.560 p= 1.000	TS= -2.100 p= 1.000	TS= -0.793 p= 1.000	TS= 2.100 p= 1.000	TS= 1.143 p= 1.000
8									-	TS= -1.540 p= 1.000	TS= -0.233 p= 1.000	TS= 2.660 p= 0.610	TS= 1.703 p= 0.092
9										-	TS= 1.306 p= 1.000	TS= 4.199 p= 0.002	TS= 3.243 p= 1.000
10											-	TS= 2.893 p= 0.298	TS= 1.936 p= 1.000
11												-	TS=-0.957 p= 1.000
12													-

Appendix D.

Kruskal Wallis post-hoc pairwise comparison with Bonferonni correction for the mineral in subaerial immature bone material. Test statistic (TS) and p-values given for the comparison between each month.

Month	*0*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
0	-	TS= 2.660 p= 0.610	TS= 2.135 p= 1.000	TS= 2.018 p= 1.000	TS= 1.353 p= 1.000	TS= 2.333 p= 1.000	TS= 1.073 p= 1.000	TS= 1.447 p= 1.000	TS= 2.916 p= 1.000	TS= 3.290 p= 0.078	TS= 2.940 p= 0.256	TS= -0.047 p= 1.000	TS= -0.583 p= 1.000
1		-	TS= -0.525 p= 1.000	TS= -0.642 p= 1.000	TS= -1.307 p= 1.000	TS= -0.327 p= 1.000	TS= -1.587 p= 1.000	TS= -1.213 p= 1.000	TS= 0.257 p= 1.000	TS= 0.630 p= 1.000	TS= 0.280 p= 1.000	TS= -2.706 p= 0.531	TS= -3.243 p= 0.092
2			-	TS= -0.117 p= 1.000	TS= -0.782 p= 1.000	TS= 0.198 p= 1.000	TS= -1.062 p= 1.000	TS= -0.668 p= 1.000	TS= 0.782 p= 1.000	TS= 1.155 p= 1.000	TS= 0.805 p= 1.000	TS= -2.181 p= 1.000	TS= -2.718 p= 0.512
3				-	TS= -0.665 p= 1.000	TS= 0.315 p= 1.000	TS= -0.945 p= 1.000	TS= -0.572 p= 1.000	TS= 0.898 p= 1.000	TS= 1.272 p= 1.000	TS= 0.922 p= 1.000	TS= -2.065 p= 1.000	TS= -2.601 p= 0.724
4					-	TS= 0.980 p= 1.000	TS= -0.280 p= 1.000	TS= 0.093 p= 1.000	TS= 1.563 p= 1.000	TS= 1.936 p= 1.000	TS= 1.587 p= 1.000	TS= -1.400 p= 1.000	TS= -1.936 p= 1.000
5						-	TS= -1.260 p= 1.000	TS= -0.887 p= 1.000	TS= 0.583 p= 1.000	TS= 0.957 p= 1.000	TS= 0.607 p= 1.000	TS= -2.380 p= 1.000	TS= -2.916 p= 0.276
6							-	TS= 0.373 p= 1.000	TS= 1.843 p= 1.000	TS= 2.216 p= 1.000	TS= 1.866 p= 1.000	TS= -1.120 p= 1.000	TS= -1.657 p= 1.000
7								-	TS= 1.470 p= 1.000	TS= 1.843 p= 1.000	TS= 1.493 p= 1.000	TS= -1.493 p= 1.000	TS= -2.030 p= 1.000
8									-	TS= 0.373 p= 1.000	TS=0.023 p= 1.000	TS= -2.963 p= 0.238	TS= -3.500 p= 0.036
9										-	TS= -0.350 p= 1.000	TS=-3.336 p= 0.066	TS=-3.873 p= 0.008
10											-	TS= -2.986 p= 0.220	TS= -3.523 p= 0.033
11												-	TS= -0.537 p= 1.000
12													-

Appendix E.

Kruskal Wallis post-hoc pairwise comparison with Bonferonni correction for the unbound water in subaerial mature bone material. Test statistic (TS) and p-values given for the comparison between each month.

Month	*0*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
0	-	TS= 1.283 p= 1.000	TS= -1.353 p= 1.000	TS= -1.143 p= 1.000	TS= -2.403 p= 1.000	TS= -2.450 p= 1.000	TS=0.163 p= 1.000	TS= 0.117 p= 1.000	TS= -2.916 p= 0.276	TS= -0.840 p= 1.000	TS= 1.026 p= 1.000	TS= -1.143 p= 1.000	TS= 0.257 p= 1.000
1		-	TS= -2.636 p= 0.654	TS= -2.426 p= 1.000	TS= -3.686 p= 0.018	TS= -3.733 p= 0.015	TS= -1.120 p= 1.000	TS=-1.166 p= 1.000	TS= -4.199 p= 0.002	TS= -2.123 p= 1.000	TS= -0.257 p= 1.000	TS= -2.426 p= 1.000	TS= -1.026 p= 1.000
2			-	TS= 0.210 p= 1.000	TS= p= 1.000	TS= -1.096 p= 1.000	TS= 1.516 p= 1.000	TS= 1.470 p= 1.000	TS= -1.563 p= 1.000	TS= 0.513 p= 1.000	TS= 2.380 p= 1.000	TS= 0.210 p= 1.000	TS= 1.610 p= 1.000
3				-	TS= -1.260 p= 1.000	TS= -1.306 p= 1.000	TS= 1.306 p= 1.000	TS=1.260 p= 1.000	TS= -1.773 p= 1.000	TS= 0.303 p= 1.000	TS= 2.170 p= 1.000	TS= 0.000 p= 1.000	TS= 1.400 p= 1.000
4					-	TS= -0.047 p= 1.000	TS= 2.566 p= 0.802	TS= 2.520 p= 0.916	TS= -0.513 p= 1.000	TS= 1.563 p= 1.000	TS= 3.429 p= 0.047	TS= 1.260 p= 1.000	TS= 2.660 p= 0.610
5						-	TS= 2.613 p= 0.700	TS= 2.566 p= 0.802	TS= -0.467 p= 1.000	TS= 1.610 p= 1.000	TS= 3.476 p= 0.040	TS= 1.306 p= 1.000	TS= 2.706 p= 0.531
6							-	TS= -0.047 p= 1.000	TS= -3.079 p= 0.162	TS= -1.003 p= 1.000	TS= 0.863 p= 1.000	TS= -1.306 p= 1.000	TS= 0.093 p= 1.000
7								-	TS= -3.033 p= 0.189	TS=-0.957 p= 1.000	TS= 0.910 p= 1.000	TS= -1.260 p= 1.000	TS= 0.140 p= 1.000
8									-	TS= 2.076 p= 1.000	TS= 3.943 p= 0.006	TS= 1.773 p= 1.000	TS= 3.173 p= 0.118
9										-	TS= 1.866 p= 1.000	TS= -0.303 p= 1.000	TS= 1.096 p= 1.000
10											-	TS= -2.170 p= 1.000	TS= -0.770 p= 1.000
11												-	TS= 1.400 p= 1.000
12													-

Appendix F.

Kruskal Wallis post-hoc pairwise comparison with Bonferonni correction for the bound water in subaerial mature bone material. Test statistic (TS) and p-values given for the comparison between each month.

Month	*0*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
0	-	TS= -1.376 p= 1.000	TS= 0.723 p= 1.000	TS= 0.933 p= 1.000	TS= -2.240 p= 1.000	TS= -2.426 p= 1.000	TS= -1.540 p= 1.000	TS= -2.613 p= 1.000	TS= -1.680 p= 1.000	TS= -2.076 p= 1.000	TS= -2.333 p= 1.000	TS= -0.443 p= 1.000	TS= 0.513 p= 1.000
1		-	TS= 2.100 p= 1.000	TS= 2.310 p= 1.000	TS= -0.863 p= 1.000	TS= -1.050 p= 1.000	TS= -0.163 p= 1.000	TS= -1.236 p= 1.000	TS= -0.303 p= 1.000	TS= -0.700 p= 1.000	TS= -0.957 p= 1.000	TS= 0.933 p= 1.000	TS= 1.890 p= 1.000
2			-	TS= 0.210 p= 1.000	TS= -2.963 p= 0.238	TS= -3.149 p= 0.128	TS= -2.263 p= 1.000	TS= -3.336 p= 0.066	TS= -2.403 p= 1.000	TS= -2.800 p= 0.399	TS= -3.056 p= 0.175	TS= -1.166 p= 1.000	TS= -0.210 p= 1.000
3				-	TS= -3.173 p= 0.118	TS= -3.359 p= 0.061	TS= -2.473 p= 1.000	TS= -3.546 p= 0.030	TS= -2.613 p= 0.700	TS= -3.010 p= 0.204	TS= -3.266 p= 0.085	TS= -1.376 p= 1.000	TS= -0.420 p= 1.000
4					-	TS= -0.187 p= 1.000	TS= 0.700 p= 1.000	TS= -0.373 p= 1.000	TS= 0.560 p= 1.000	TS= 0.163 p= 1.000	TS= -0.093 p= 1.000	TS= 1.796 p= 1.000	TS= 2.753 p= 0.461
5						-	TS= 0.887 p= 1.000	TS= -0.187 p= 1.000	TS= 0.747 p= 1.000	TS= 0.350 p= 1.000	TS= 0.093 p= 1.000	TS= 1.983 p= 1.000	TS=2.940 p= 0.256
6							-	TS= -1.073 p= 1.000	TS= -0.140 p= 1.000	TS= -0.537 p= 1.000	TS= -0.793 p= 1.000	TS= 1.096 p= 1.000	TS= 2.053 p= 1.000
7								-	TS= 0.933 p= 1.000	TS= 0.537 p= 1.000	TS= 0.280 p= 1.000	TS= 2.170 p= 1.000	TS= 3.126 p= 0.138
8									-	TS= -0.397 p= 1.000	TS= -0.653 p= 1.000	TS= 1.236 p= 1.000	TS= 2.193 p= 1.000
9										-	TS= -0.257 p= 1.000	TS= 1.633 p= 1.000	TS= 2.590 p= 0.750
10											-	TS= 1.890 p= 1.000	TS= 2.846 p= 0.345
11												-	TS= 0.957 p= 1.000
12													-

Appendix G.

Kruskal Wallis post-hoc pairwise comparison with Bonferonni correction for the collagen in subaerial mature bone material. Test statistic (TS) and p-values given for the comparison between each month.

Month	*0*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
0	-	TS= -2.018 p= 1.000	TS= -1.995 p= 1.000	TS= -1.027 p= 1.000	TS= -0.852 p= 1.000	TS= -1.575 p= 1.000	TS= -2.998 p= 0.212	TS= -2.578 p= 0.775	TS= -3.651 p= 0.020	TS= -3.091 p= 0.155	TS= -3.371 p= 0.058	TS= -1.948 p= 1.000	TS= -2.345 p= 1.000
1		-	TS= 0.023 p= 1.000	TS= 0.992 p= 1.000	TS= 0.243 p= 1.000	TS= 0.443 p= 1.000	TS= -0.980 p= 1.000	TS= -0.560 p= 1.000	TS= -1.633 p= 1.000	TS= -1.073 p= 1.000	TS=-1.353 p= 1.000	TS= 0.070 p= 1.000	TS= -0.327 p= 1.000
2			-	TS= 0.968 p= 1.000	TS= 1.143 p= 1.000	TS= 0.420 p= 1.000	TS= -1.003 p= 1.000	TS= -0.583 p= 1.000	TS= -1.656 p= 1.000	TS= -1.097 p= 1.000	TS= -1.376 p= 1.000	TS= 0.047 p= 1.000	TS= -0.350 p= 1.000
3				-	TS= 0.175 p= 1.000	TS= -0.548 p= 1.000	TS= -1.971 p= 1.000	TS= -1.551 p= 1.000	TS= -2.625 p= 0.677	TS= -2.065 p= 1.000	TS= -2.345 p= 1.000	TS= -0.922 p= 1.000	TS=-1.318 p= 1.000
4					-	TS= -0.723 p= 1.000	TS= -2.146 p= 1.000	TS= -1.726 p= 1.000	TS= -2.800 p= 0.399	TS= -2.240 p= 1.000	TS= -2.520 p= 0.916	TS= -1.097 p= 1.000	TS= -1.493 p= 1.000
5						-	TS= -1.423 p= 1.000	TS= -1.003 p= 1.000	TS= -2.076 p= 1.000	TS= -1.516 p= 1.000	TS= -1.796 p= 1.000	TS= -0.373 p= 1.000	TS= -0.770 p= 1.000
6							-	TS= 0.420 p= 1.000	TS= -0.653 p= 1.000	TS= -0.093 p= 1.000	TS= -0.373 p= 1.000	TS= 1.050 p= 1.000	TS= 0.653 p= 1.000
7								-	TS= -1.073 p= 1.000	TS= -0.513 p= 1.000	TS= -0.793 p= 1.000	TS= 0.630 p= 1.000	TS= 0.233 p= 1.000
8									-	TS= 0.560 p= 1.000	TS= 0.280 p= 1.000	TS= 1.703 p= 1.000	TS= 1.306 p= 1.000
9										-	TS= -0.280 p= 1.000	TS= 1.143 p= 1.000	TS= 0.747 p= 1.000
10											-	TS= 1.423 p= 1.000	TS= 1.027 p= 1.000
11												-	TS= -0.397 p= 1.000
12													-

Appendix H.

Kruskal Wallis post-hoc pairwise comparison with Bonferonni correction for the mineral in subaerial mature bone material. Test statistic (TS) and p-values given for the comparison between each month.

Month	*0*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
0	-	TS= 1.213 p= 1.000	TS= 1.866 p= 1.000	TS= 0.887 p= 1.000	TS= 2.310 p= 1.000	TS= 2.706 p= 0.531	TS= 2.683 p= 0.569	TS= 2.543 p= 0.857	TS= 4.199 p= 0.002	TS= 3.033 p= 0.189	TS= 2.893 p= 0.298	TS= 1.983 p= 1.000	TS= 1.283 p= 1.000
1		-	TS= 0.653 p= 1.000	TS= -0.327 p= 1.000	TS= 1.096 p= 1.000	TS= 1.493 p= 1.000	TS= 1.470 p= 1.000	TS= 1.330 p= 1.000	TS= 2.986 p= 0.072	TS= 1.820 p= 1.000	TS= 1.680 p= 1.000	TS= 0.770 p= 1.000	TS= 0.070 p= 1.000
2			-	TS= -0.980 p= 1.000	TS= 0.443 p= 1.000	TS= 0.840 p= 1.000	TS= 0.817 p= 1.000	TS= 0.677 p= 1.000	TS= 2.333 p= 1.000	TS= 1.166 p= 1.000	TS= 1.026 p= 1.000	TS= 0.117 p= 1.000	TS= -0583 p= 1.000
3				-	TS= 1.423 p= 1.000	TS= 1.820 p= 1.000	TS= 1.796 p= 1.000	TS= 1.656 p= 1.000	TS= 3.313 p= 0.072	TS= 2.146 p= 1.000	TS= 2.006 p= 1.000	TS= 1.096 p= 1.000	TS= 0.397 p= 1.000
4					-	TS= 0.397 p= 1.000	TS= 0.373 p= 1.000	TS= 0.233 p= 1.000	TS= 1.890 p= 1.000	TS= 0.723 p= 1.000	TS= 0.583 p= 1.000	TS= -0.327 p= 1.000	TS= -1.026 p= 1.000
5						-	TS= -0.023 p= 1.000	TS= -0.163 p= 1.000	TS= 1.493 p= 1.000	TS= 0.327 p= 1.000	TS= 0.187 p= 1.000	TS= -0.723 p= 1.000	TS= -1.423 p= 1.000
6							-	TS= -0.140 p= 1.000	TS= 1.516 p= 1.000	TS= 0.350 p= 1.000	TS= 0.210 p= 1.000	TS= -0.700 p= 1.000	TS= -1.400 p= 1.000
7								-	TS= 1.656 p= 1.000	TS= 0.490 p= 1.000	TS= p= 1.000	TS= -0.560 p= 1.000	TS= -1.260 p= 1.000
8									-	TS= -1.166 p= 1.000	TS= -1.306 p= 1.000	TS= -2.216 p= 1.000	TS= -2.916 p= 0.276
9										-	TS= -0.140 p= 1.000	TS= -1.050 p= 1.000	TS= -1.750 p= 1.000
10											-	TS= -0.910 p= 1.000	TS= -1.610 p= 1.000
11												-	TS= -0l700 p= 1.000
12													-

Appendix I.

Kruskal Wallis post-hoc pairwise comparison with Bonferonni correction for the unbound water in buried immature bone material. Test statistic (TS) and p-values given for the comparison between each month.

Month	*0*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
0	-	TS= -0.443 p= 1.000	TS= -0.957 p= 1.000	TS= -1.983 p= 1.000	TS= -2.753 p= 0.461	TS= -2.146 p= 1.000	TS= -1.586 p= 1.000	TS= -2.858 p= 0.333	TS= -4.339 p= 0.001	TS= -4.153 p= 0.003	TS= -3.208 p= 0.104	TS= -3.430 p= 0.047	TS= -0.653 p= 1.000
1		-	TS= -0.513 p= 1.000	TS= -1.540 p= 1.000	TS= -2.310 p= 1.000	TS= -1.703 p= 1.000	TS= -1.143 p= 1.000	TS= p= 1.000	TS= -3.896 p= 0.008	TS= -3.709 p= 0.016	TS= -2.765 p= 0.445	TS= -2.986 p= 0.220	TS= -0.210 p= 1.000
2			-	TS= -1.027 p= 1.000	TS= -1.796 p= 1.000	TS= -1.190 p= 1.000	TS= -0.630 p= 1.000	TS= -1.901 p= 1.000	TS= -3.383 p= 0.056	TS= -3.196 p= 0.109	TS= -2.251 p= 1.000	TS= -2.473 p= 1.000	TS= 0.303 p= 1.000
3				-	TS= -0.770 p= 1.000	TS= -0.163 p= 1.000	TS= 0.397 p= 1.000	TS= -0.875 p= 1.000	TS= -2.356 p= 1.000	TS= -2.170 p= 1.000	TS= -1.225 p= 1.000	TS= -1.446 p= 1.000	TS= 1.330 p= 1.000
4					-	TS= 0.607 p= 1.000	TS= 1.167 p= 1.000	TS= -0.105 p= 1.000	TS= -1.586 p= 1.000	TS= -1.400 p= 1.000	TS= -0.455 p= 1.000	TS= -0.677 p= 1.000	TS= 2.100 p= 1.000
5						-	TS= 0.560 p= 1.000	TS= -0.712 p= 1.000	TS= -2.193 p= 1.000	TS= -2.006 p= 1.000	TS= -1.062 p= 1.000	TS= -1.283 p= 1.000	TS= 1.493 p= 1.000
6							-	TS= -1.271 p= 1.000	TS= -2.753 p= 0.461	TS= -2.566 p= 0.802	TS= -1.621 p= 1.000	TS= -1.842 p= 1.000	TS= 0.933 p= 1.000
7								-	TS= -1.481 p= 1.000	TS= -1.295 p= 1.000	TS= -0.350 p= 1.000	TS= -0.572 p= 1.000	TS= 2.205 p= 1.000
8									-	TS= 0.187 p= 1.000	TS= 1.132 p= 1.000	TS= 0.910 p= 1.000	TS= 3.686 p= 0.018
9										-	TS= 0.945 p= 1.000	TS= 0.723 p= 1.000	TS= 3.500 p= 0.036
10											-	TS= -0.222 p= 1.000	TS= 2.555 p= 0.829
11												-	TS= 2.776 p= 0.429
12													-

Appendix J.

Kruskal Wallis post-hoc pairwise comparison with Bonferonni correction for the bound water in buried immature bone material. Test statistic (TS) and p-values given for the comparison between each month.

Month	*0*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
0	-	TS= -0.723 p= 1.000	TS= 0.840 p= 1.000	TS= -1.481 p= 1.000	TS= -2.543 p= 0.857	TS= -1.843 p= 1.000	TS= -1.796 p= 1.000	TS= -2.473 p= 1.000	TS= -1.621 p= 1.000	TS= -0.420 p= 1.000	TS= 0.327 p= 1.000	TS= 0.817 p= 1.000	TS= 1.516 p= 1.000
1		-	TS= 1.562 p= 1.000	TS= -0.758 p= 1.000	TS= -1.820 p= 1.000	TS= -1.120 p= 1.000	TS= -1.073 p= 1.000	TS= -1.750 p= 1.000	TS= -0.898 p= 1.000	TS= 0.303 p= 1.000	TS= 1.050 p= 1.000	TS= 1.540 p= 1.000	TS= 2.240 p= 1.000
2			-	TS= -2.321 p= 1.000	TS= -3.383 p= 0.056	TS= -2.683 p= 0.569	TS= -2.636 p= 0.654	TS= -3.313 p= 0.072	TS= -2.461 p= 1.000	TS= -1.260 p= 1.000	TS= -0.513 p= 1.000	TS= -0.023 p= 1.000	TS= 0.677 p= 1.000
3				-	TS= -1.062 p= 1.000	TS= -0.362 p= 1.000	TS= -0.315 p= 1.000	TS= -0.992 p= 1.000	TS= -0.140 p= 1.000	TS= 1.062 p= 1.000	TS= 1.808 p= 1.000	TS= 2.298 p= 1.000	TS= 2.998 p= 0.212
4					-	TS= 0.700 p= 1.000	TS= 0.747 p= 1.000	TS= 0.070 p= 1.000	TS= 0.922 p= 1.000	TS= 2.123 p= 1.000	TS= 2.870 p= 0.321	TS= 3.360 p= 1.000	TS= 4.059 p= 0.004
5						-	TS= 0.047 p= 1.000	TS= -0.630 p= 1.000	TS= 0.222 p= 1.000	TS= 1.423 p= 1.000	TS= 2.170 p= 1.000	TS= 2.660 p= 0.610	TS= 3.360 p= 0.061
6							-	TS= -0.677 p= 1.000	TS= 0.175 p= 1.000	TS= 1.376 p= 1.000	TS= 2.123 p= 1.000	TS= 2.613 p= 0.700	TS= 3.313 p= 0.072
7								-	TS= 0.852 p= 1.000	TS= 2.053 p= 1.000	TS= 2.800 p= 0.399	TS= 3.290 p= 1.000	TS= 3.989 p= 0.005
8									-	TS= 1.201 p= 1.000	TS= 1.948 p= 1.000	TS= 2.438 p= 1.000	TS= 3.138 p= 0.133
9										-	TS= 0.747 p= 1.000	TS= 1.236 p= 1.000	TS= 1.936 p= 1.000
10											-	TS= 0.490 p= 1.000	TS= 1.190 p= 1.000
11												-	TS= 0.700 p= 1.000
12													-

Appendix K.

Kruskal Wallis post-hoc pairwise comparison with Bonferonni correction for the collagen in buried immature bone material. Test statistic (TS) and p-values given for the comparison between each month.

Month	*0*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
0	-	TS= 1.073 p= 1.000	TS= 0.700 p= 1.000	TS= 2.263 p= 1.000	TS= 4.433 p= 0.001	TS= 2.660 p= 1.000	TS= 3.313 p= 0.072	TS= 1.820 p= 1.000	TS= 2.590 p= 0.750	TS= 1.213 p= 1.000	TS= 1.470 p= 1.000	TS= 3.943 p= 0.006	TS= 3.639 p= 0.021
1		-	TS= -0.373 p= 1.000	TS= 1.190 p= 1.000	TS= 3.359 p= 0.061	TS= 1.586 p= 1.000	TS= 2.240 p= 1.000	TS= 0.747 p= 1.000	TS= 1.516 p= 1.000	TS= 0.140 p= 1.000	TS= 0.397 p= 1.000	TS= 2.870 p= 0.321	TS= 2.566 p= 0.802
2			-	TS= 1.563 p= 1.000	TS= 3.733 p= 0.015	TS= 1.960 p= 1.000	TS= 2.613 p= 0.700	TS= 1.120 p= 1.000	TS= 1.890 p= 1.000	TS= 0.513 p= 1.000	TS= 0.770 p= 1.000	TS= 3.243 p= 0.092	TS= 2.940 p= 0.256
3				-	TS= 2.170 p= 1.000	TS= 0.397 p= 1.000	TS= 1.050 p= 1.000	TS= -0.443 p= 1.000	TS= 0.327 p= 1.000	TS= -1.050 p= 1.000	TS= -0.793 p= 1.000	TS= 1.680 p= 1.000	TS= 1.376 p= 1.000
4					-	TS= -1.773 p= 1.000	TS= -1.120 p= 1.000	TS= -2.613 p= 0.700	TS= -1.843 p= 1.000	TS= -3.219 p= 0.100	TS= -2.963 p= 0.238	TS= -0.490 p= 1.000	TS= -0.793 p= 1.000
5						-	TS= 0.653 p= 1.000	TS= -0.840 p= 1.000	TS= -0.070 p= 1.000	TS= -1.446 p= 1.000	TS= -1.190 p= 1.000	TS= 1.283 p= 1.000	TS= 0.980 p= 1.000
6							-	TS= -1.493 p= 1.000	TS= -0.723 p= 1.000	TS= -2.100 p= 1.000	TS= -1.843 p= 1.000	TS= 0.630 p= 1.000	TS= 0.327 p= 1.000
7								-	TS= 0.770 p= 1.000	TS= -0.607 p= 1.000	TS= -0.350 p= 1.000	TS= 2.123 p= 1.000	TS= 1.820 p= 1.000
8									-	TS= -1.376 p= 1.000	TS= -1.120 p= 1.000	TS= 1.353 p= 1.000	TS= 1.050 p= 1.000
9										-	TS= 0.257 p= 1.000	TS= 2.730 p= 0.495	TS= 2.426 p= 1.000
10											-	TS= 2.473 p= 1.000	TS= 2.170 p= 1.000
11												-	TS= -0.303 p= 1.000
12													-

Appendix L.

Kruskal Wallis post-hoc pairwise comparison with Bonferonni correction for the mineral in buried immature bone material. Test statistic (TS) and p-values given for the comparison between each month.

Month	*0*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
0	-	TS= -2.590 p= 0.749	TS= -2.181 p= 1.000	TS= -2.461 p= 1.000	TS= -3.686 p= 0.018	TS= -2.461 p= 0.979	TS= -3.360 p= 0.061	TS= -1.423 p= 1.000	TS= -0.747 p= 1.000	TS= -0630 p= 1.000	TS= -1.376 p= 1.000	TS= -3.686 p= 0.018	TS= -4.479 p= 0.001
1		-	TS= 0.408 p= 1.000	TS= 0.128 p= 1.000	TS= -1.097 p= 1.000	TS= 0.093 p= 1.000	TS= -0.770 p= 1.000	TS= 1.167 p= 1.000	TS= 1.843 p= 1.000	TS= 1.960 p= 1.000	TS= 1.213 p= 1.000	TS= -1.097 p= 1.000	TS= -1.890 p= 1.000
2			-	TS= -0.280 p= 1.000	TS= -1.505 p= 1.000	TS= -0.315 p= 1.000	TS= -1.178 p= 1.000	TS= 0.758 p= 1.000	TS= 1.435 p= 1.000	TS= 1.551 p= 1.000	TS= 0.805 p= 1.000	TS= -1.505 p= 1.000	TS= -2.298 p= 1.000
3				-	TS= -1.225 p= 1.000	TS= -0.035 p= 1.000	TS= -0.898 p= 1.000	TS= 1.038 p= 1.000	TS= 1.715 p= 1.000	TS= 1.831 p= 1.000	TS= 1.085 p= 1.000	TS= -1.225 p= 1.000	TS= -2.018 p= 1.000
4					-	TS= 1.190 p= 1.000	TS= 0.327 p= 1.000	TS= 2.263 p= 1.000	TS= 2.940 p= 0.256	TS= 3.056 p= 0.175	TS= 2.310 p= 1.000	TS= 0.000 p= 1.000	TS= -0.793 p= 1.000
5						-	TS= -0.863 p= 1.000	TS= 1.073 p= 1.000	TS= 1.750 p= 1.000	TS= 1.866 p= 1.000	TS= 1.120 p= 1.000	TS= -1.190 p= 1.000	TS= -1.983 p= 1.000
6							-	TS= 1.936 p= 1.000	TS= 2.613 p= 0.700	TS= 2.730 p= 0.495	TS= 1.983 p= 1.000	TS= -0.327 p= 1.000	TS= -1.120 p= 1.000
7								-	TS= 0.677 p= 1.000	TS= 0.793 p= 1.000	TS= 0.047 p= 1.000	TS= -2.263 p= 1.000	TS= -3.056 p= 1.000
8									-	TS= 0.117 p= 1.000	TS= -0.630 p= 1.000	TS= -2.940 p= 0.256	TS= -3.733 p= 0.015
9										-	TS= -0.747 p= 1.000	TS= -3.056 p= 0.175	TS= -3.849 p= 0.009
10											-	TS= -2.301 p= 1.000	TS= -3.103 p= 0.149
11												-	TS= -0.793 p= 1.000
12													-

Appendix M.

Kruskal Wallis post-hoc pairwise comparison with Bonferonni correction for the unbound water in buried mature bone material. Test statistic (TS) and p-values given for the comparison between each month.

Month	*0*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
0	-	TS= 1.190 p= 1.000	TS= -0.560 p= 1.000	TS= 0.245 p= 1.000	TS= -0.467 p= 1.000	TS= -2.100 p= 1.000	TS= -0.397 p= 1.000	TS= -1.446 p= 1.000	TS= -3.290 p= 0.078	TS= -2.870 p= 0.321	TS= -1.586 p= 1.000	TS= -2.496 p= 0.979	TS= -.128 p= 1.000
1		-	TS= -1.750 p= 1.000	TS= -0.945 p= 1.000	TS= -1.656 p= 1.000	TS= -3.290 p= 0.018	TS= -1.586 p= 1.000	TS= -2.636 p= 0.654	TS= -4.479 p= 0.001	TS= -4.059 p= 0.004	TS= -2.776 p= 0.429	TS= -3.686 p= 0.018	TS= -1.062 p= 1.000
2			-	TS= 0.805 p= 1.000	TS= 0.093 p= 1.000	TS= -1.540 p= 1.000	TS= 0.163 p= 1.000	TS= -0.887 p= 1.000	TS= -2.730 p= 0.495	TS= -2.310 p= 1.000	TS= -1.027 p= 1.000	TS= -1.936 p= 1.000	TS= 0.688 p= 1.000
3				-	TS= -0.712 p= 1.000	TS= -2.345 p= 1.000	TS= -0.642 p= 1.000	TS= -1.691 p= 1.000	TS= -3.534 p= 0.032	TS= -3.115 p= 0.144	TS= -1.831 p= 1.000	TS= -2.741 p= 0.477	TS= -0.117 p= 1.000
4					-	TS= -1.633 p= 1.000	TS= 0.070 p= 1.000	TS= -0.980 p= 1.000	TS= -2.823 p= 0.371	TS= -2.403 p= 1.000	TS= -1.120 p= 1.000	TS= -2.030 p= 1.000	TS= 0.595 p= 1.000
5						-	TS= 1.703 p= 1.000	TS= 0.653 p= 1.000	TS= -1.190 p= 1.000	TS= -0.770 p= 1.000	TS= 0.513 p= 1.000	TS= -0.397 p= 1.000	TS= 2.228 p= 1.000
6							-	TS= -1.050 p= 1.000	TS= -2.893 p= 0.298	TS= -2.473 p= 1.000	TS= -1.190 p= 1.000	TS= -2.100 p= 1.000	TS= 0.525 p= 1.000
7								-	TS= -1.843 p= 1.000	TS= -1.423 p= 1.000	TS= -0.140 p= 1.000	TS= -1.050 p= 1.000	TS= 1.575 p= 1.000
8									-	TS= 0.420 p= 1.000	TS= 1.703 p= 1.000	TS= 0.793 p= 1.000	TS= 3.418 p= 0.049
9										-	TS= 1.283 p= 1.000	TS= 0.373 p= 1.000	TS= 2.998 p= 0.212
10											-	TS= -0910 p= 1.000	TS= 1.715 p= 1.000
11												-	TS= 2.625 p= 0.677
12													-

Appendix N.

Kruskal Wallis post-hoc pairwise comparison with Bonferonni correction for the bound water in buried mature bone material. Test statistic (TS) and p-values given for the comparison between each month.

Month	*0*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
0	-	TS= -0.537 p= 1.000	TS= 1.073 p= 1.000	TS= 0.747 p= 1.000	TS= -1.050 p= 1.000	TS= -2.170 p= 1.000	TS= -1.376 p= 1.000	TS= -2.986 p= 0.220	TS= -1.995 p= 1.000	TS= -3.313 p= 0.072	TS= -2.240 p= 1.000	TS= -0.747 p= 1.000	TS= -0.268 p= 1.000
1		-	TS= 1.610 p= 1.000	TS= 1.283 p= 1.000	TS= -0.513 p= 1.000	TS= -1.633 p= 1.000	TS= -0.840 p= 1.000	TS= -2.450 p= 1.000	TS= -1.458 p= 1.000	TS= -2.776 p= 0.429	TS= -1.703 p= 1.000	TS= -0.210 p= 1.000	TS= 0.268 p= 1.000
2			-	TS= -0.327 p= 1.000	TS= -2.123 p= 1.000	TS= -3.243 p= 0.092	TS= -2.450 p= 1.000	TS= -4.059 p= 0.004	TS= -3.068 p= 0.168	TS= -4.386 p= 0.001	TS= -3.313 p= 0.072	TS= -1.820 p= 1.000	TS= -1.341 p= 1.000
3				-	TS= -1.796 p= 1.000	TS= -2.916 p= 0.276	TS= -2.123 p= 1.000	TS= -3.733 p= 0.015	TS= -2.741 p= 0.477	TS= -4.059 p= 0.004	TS= -2.986 p= 0.220	TS= -1.493 p= 1.000	TS= -1.015 p= 1.000
4					-	TS= -1.120 p= 1.000	TS= -0.327 p= 1.000	TS= -1.936 p= 1.000	TS= -0.945 p= 1.000	TS= -2.263 p= 1.000	TS= -1.190 p= 1.000	TS= 0.303 p= 1.000	TS= 0.782 p= 1.000
5						-	TS= 0.793 p= 1.000	TS= -0.817 p= 1.000	TS= 0.175 p= 1.000	TS= -1.143 p= 1.000	TS= -0.070 p= 1.000	TS= 1.493 p= 1.000	TS= 1.901 p= 1.000
6							-	TS= -1.610 p= 1.000	TS= -0.618 p= 1.000	TS= -1.936 p= 1.000	TS= -0.863 p= 1.000	TS= 0.630 p= 1.000	TS= 1.108 p= 1.000
7								-	TS= 0.992 p= 1.000	TS= -0.327 p= 1.000	TS= 0.747 p= 1.000	TS= 2.240 p= 1.000	TS= 2.718 p= 0.512
8									-	TS= -1.318 p= 1.000	TS= -0.245 p= 1.000	TS= 1.248 p= 1.000	TS= 1.726 p= 1.000
9										-	TS= 1.073 p= 1.000	TS= 2.566 p= 0.802	TS= 3.045 p= 0.182
10											-	TS= 1.149 p= 1.000	TS= 1.971 p= 1.000
11												-	TS= 0.478 p= 1.000
12													-

Appendix O.

Kruskal Wallis post-hoc pairwise comparison with Bonferonni correction for the collagen in buried mature bone material. Test statistic (TS) and p-values given for the comparison between each month.

Month	*0*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
0	-	TS= 3.056 p= 1.000	TS= 2.333 p= 1.000	TS= 3.943 p= 0.006	TS= 3.989 p= 0.005	TS= 1.610 p= 1.000	TS= 1.283 p= 1.000	TS= 2.846 p= 0.345	TS= 0.793 p= 1.000	TS= 2.823 p= 0.371	TS= 0.887 p= 1.000	TS= 2.520 p= 0.916	TS= 1.516 p= 1.000
1		-	TS= -0.723 p= 1.000	TS= 0.887 p= 1.000	TS= 0.933 p= 1.000	TS= -1.446 p= 1.000	TS= -1.773 p= 1.000	TS= -0.210 p= 1.000	TS= -2.263 p= 1.000	TS= -0.233 p= 1.000	TS= -2.170 p= 1.000	TS= -0.537 p= 1.000	TS= -1.540 p= 1.000
2			-	TS= 1.610 p= 1.000	TS= 1.656 p= 1.000	TS= -0.723 p= 1.000	TS= -1.050 p= 1.000	TS= 0.513 p= 1.000	TS= -1.540 p= 1.000	TS= 0.490 p= 1.000	TS= -1.446 p= 1.000	TS= 0.187 p= 1.000	TS= -0.817 p= 1.000
3				-	TS= 0.047 p= 1.000	TS= -2.333 p= 1.000	TS= -3.056 p= 1.000	TS= -1.096 p= 1.000	TS= -3.149 p= 0.128	TS= -1.120 p= 1.000	TS= p= 1.000	TS= -1.423 p= 1.000	TS= -2.426 p= 1.000
4					-	TS= -2.380 p= 1.000	TS= -2.706 p= 0.531	TS= -1.143 p= 1.000	TS= -3.196 p= 0.109	TS= -1.166 p= 1.000	TS= -3.103 p= 0.150	TS= -1.470 p= 1.000	TS= -2.473 p= 1.000
5						-	TS= -0.327 p= 1.000	TS= 1.236 p= 1.000	TS= -0.817 p= 1.000	TS= 1.213 p= 1.000	TS= -0.723 p= 1.000	TS= 0.910 p= 1.000	TS= -0.093 p= 1.000
6							-	TS= 1.563 p= 1.000	TS= -0.490 p= 1.000	TS= 1.540 p= 1.000	TS= -0.397 p= 1.000	TS= 1.236 p= 1.000	TS= 0.233 p= 1.000
7								-	TS= -2.053 p= 1.000	TS= -0.023 p= 1.000	TS= -1.960 p= 1.000	TS= -0.327 p= 1.000	TS= -1.330 p= 1.000
8									-	TS= 2.030 p= 1.000	TS= 0.093 p= 1.000	TS= 1.726 p= 1.000	TS= 0.723 p= 1.000
9										-	TS= -1.936 p= 1.000	TS= -0.303 p= 1.000	TS= -1.306 p= 1.000
10											-	TS= 1.633 p= 1.000	TS= 0.630 p= 1.000
11												-	TS= -1.003 p= 1.000
12													-

Appendix P.

Kruskal Wallis post-hoc pairwise comparison with Bonferonni correction for the mineral in buried mature bone material. Test statistic (TS) and p-values given for the comparison between each month.

Month	*0*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
0	-	TS= -3.359 p= 0.061	TS= -2.753 p= 0.461	TS= -3.383 p= 0.056	TS= -2.916 p= 0.276	TS= -0.537 p= 1.000	TS= -1.213 p= 1.000	TS= -1.750 p= 1.000	TS= 0.723 p= 1.000	TS= -0.537 p= 1.000	TS= -0.350 p= 1.000	TS= -1.260 p= 1.000	TS= -1.773 p= 1.000
1		-	TS= 0.607 p= 1.000	TS= -0.023 p= 1.000	TS= 0.443 p= 1.000	TS= 2.823 p= 0.371	TS= 2.146 p= 1.000	TS= 1.610 p= 1.000	TS= 4.083 p= 0.003	TS= 2.823 p= 0.371	TS= 3.010 p= 0l204	TS= 2.100 p= 1.000	TS= 1.586 p= 1.000
2			-	TS= -0.630 p= 1.000	TS= -0.163 p= 1.000	TS= 2.216 p= 1.000	TS= 1.540 p= 1.000	TS= 1.003 p= 1.000	TS= 3.476 p= 0.040	TS= 2.216 p= 1.000	TS= 2.403 p= 1.000	TS= 1.493 p= 1.000	TS= 0.980 p= 1.000
3				-	TS= 0.467 p= 1.000	TS= 2.846 p= 0.345	TS= 2.170 p= 1.000	TS= 1.633 p= 1.000	TS= 4.106 p= 0.003	TS= 2.846 p= 1.000	TS= 3.033 p= 0.189	TS= 2.123 p= 1.000	TS= 1.610 p= 1.000
4					-	TS= 2.380 p= 1.000	TS= 1.703 p= 1.000	TS= 1.166 p= 1.000	TS= 3.639 p= 0.021	TS= 2.380 p= 1.000	TS= 2.566 p= 0.802	TS= 1.656 p= 1.000	TS= 1.143 p= 1.000
5						-	TS= -0.677 p= 1.000	TS= -1.213 p= 1.000	TS= 1.260 p= 1.000	TS= 0.000 p= 1.000	TS= 0.187 p= 1.000	TS= -0.723 p= 1.000	TS= -1.236 p= 1.000
6							-	TS= -0.537 p= 1.000	TS= 1.936 p= 1.000	TS= 0.877 p= 1.000	TS= 0.863 p= 1.000	TS= -0.047 p= 1.000	TS= -0.560 p= 1.000
7								-	TS= 2.473 p= 1.000	TS= 1.213 p= 1.000	TS= 1.400 p= 1.000	TS= 0.490 p= 1.000	TS= -0.023 p= 1.000
8									-	TS= -1.260 p= 1.000	TS= -1.073 p= 1.000	TS= -1.983 p= 1.000	TS= -2.496 p= 0.979
9										-	TS= 0.187 p= 1.000	TS= -0.723 p= 1.000	TS= -1.236 p= 1.000
10											-	TS= -0.910 p= 1.000	TS= -1.423 p= 1.000
11												-	TS= -0.513 p= 1.000
12													-