BACK IN THE CYCLE:
A REVIEW OF THE TAPHONOMY OF BIOMINERALISED TISSUES

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Declaration

I declare that this thesis does not contain any material submitted previously for the award of any other degree or diploma at any university or other tertiary institution. Furthermore, to the best of my knowledge, it does not contain any material previously published or written by another individual, except where due reference has been made in the text. Finally, I declare that all reported experimentations performed in this research were carried out by myself, except that any contribution by others, with whom I have worked is explicitly acknowledged.

Signed: Ashleigh Lutze

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Part One

Literature Review

Back in the cycle:

a review of the taphonomy of biomineralised tissues

Title: Back in the Cycle: a review of the taphonomy of biomineralized tissue

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Abstract

After death, bones and teeth, biomineralized tissues composed of an organic and an inorganic part, are often the last remaining structures of an organism. The processes that affect biological tissues post-mortem is collectively defined as taphonomy. Forensic taphonomy studies the post-mortem modifications of remains in relation to a variety of physical, chemical, or biological agents, with the aim of assisting forensic investigations. Current research has typically focused on taphonomic effects observed in single depositional environments. This review summarizes the to-date information on the known taphonomic agents present across five depositional environments (burial, subaerial exposure, aquatic environments, burnt and frozen remains), and the effects generated on biomineralized tissues. Taphonomy is a relatively new sub-discipline of forensic anthropology and includes several areas where research is limited, such as the taphonomic processes in frozen and aquatic environments and the post-mortem alterations of teeth. As more research is conducted, the benefit of incorporating forensic taphonomy into forensic investigations have become increasingly evident. Each depositional environment features a range of characteristic taphonomic effects, which may be used to generate an accurate description of the post-mortem histories of remains. By providing training in forensic taphonomy investigative techniques, and incorporating them into investigations, more precise information may be gathered, potentially leading to faster turnaround times and case resolutions. The information presented in this review will prove useful in assisting the forensic community and may stimulate future research.

Keywords: forensic, anthropology, decomposition, bone, teeth, diagenesis, osteoarchaeology

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List of Abbreviations

BA	Bioapatite
Cm	Centimetre
DNA	Deoxyribonucleic acid
Ft	Foot
HA	Hydroxyapatite
In	Inch
IR	Infra-red
Lb	Pound
Mm	Millimetre
mtDNA	Mitochondrial DNA
PMI	Post-mortem interval
PMSI	Post-mortem submersion interval
RBCs	Red blood cells
REEs	Rare earth elements
SEM	Scanning electron microscope/microscopy
UV	Ultra-violet
µm	Micrometre/micron
CO_2	Carbon dioxide
O_2	Dioxide
°C	Degrees Celsius

1. INTRODUCTION

1.1 Biomineralized tissue

Biomineralization is a complex process which results in the formation of structured organic-inorganic tissues, such as bone and teeth, whereby mineral crystals (inorganic) are deposited into an organic matrix produced in living organisms. Bone and teeth are formed during foetal development and continue to be reproduced throughout life in response to growth stimuli and trauma. They also perform essential functions, such as providing structural and mechanical support, participating in the storage and exchange of minerals, and metabolic regulation (1, 2).

1.2 Macro- and micro-anatomy of bio-mineralized tissue

1.2.1 Bone

Bone is one of the hardest substances in the human body (3), it can withstand large tensile forces and repair itself when damaged (4). Bone is typically characterised as a specialised form of connective tissue, consisting of an organic component (mostly type I collagen) and an inorganic (or mineral) component in the form of crystals of bioapatite (BA), a type of hydroxyapatite (HA) containing carbonic acid (5) (6). HA consists primarily of calcium and phosphate (49).

Within the human body, bone performs four main functions: structural support for the body, motion at the joints by acting as levers powered by attached muscles, protection of vital organs and soft tissues from damage, and as centres for growth as the human body progresses from infancy to adulthood (7). Bones also act as mineral reservoirs for

the storage of calcium, and some house bone marrow, a vital erythropoietic tissue (7). The exterior of all bones is composed of a dense, compact bone known as cortical. It often appears smooth due to the lamellar tissue it is composed of, which is laid down in thin layers running parallel to the long axis of the bone (8). The second type of bone is known as trabecular, spongy, or cancellous, and is often found in the ends of long bones, within vertebral bodies and ribs, and within all bones of the hands and feet, among a few other anatomical locations (8). Trabecular bone is very light and porous in comparison to cortical bone and appears like a spiderweb or honeycomb structure (3), with its primary function being to provide structural support to the bone without adding excess weight (8).

1.2.2 Teeth

Teeth essentially consist of the same inorganic and organic components as bone, though their mineral content is much higher (9). Teeth are embedded into the alveolar processes of the mandible and maxilla ("jaw bones") and are formed during foetal development. Humans have two sets of teeth, referred to as deciduous teeth ("baby teeth") which are lost and are substituted by permanent teeth ("adult teeth") as the individual ages (5). There are four morphological tooth types in humans: incisors, canines, premolars, and molars, all composed of the three main tissues of enamel, dentin and cementum (10). Enamel is around 99% mineral and covers the crown, the visible part of the tooth. Dentin sits underneath the enamel, making up the bulk of the tooth, and is around 75% mineral. Cementum is approximately 65% mineral and contributes in holding the root, the hidden part of the tooth, attached to the jaw (9).

The primary function of teeth is to provide a means of mechanical digestion through the mastication of food (8). The high mineral content of teeth, which is higher than that of bone, is responsible for their excellent preservation after death and decomposition, enabling post-mortem identification and taphonomic reconstructions (9).

1.3 The decomposition process

Before biomineralized tissues become exposed to their depositional environments and can be analysed, the human body undergoes a process known as decomposition. This process commences as soon as the individual has died, that is when the vital functions, such as breathing and circulation, have irreversibly ceased (11, 12). Following death, the human body progresses through five broad stages of decomposition, each defined by a relative period: fresh, autolysis, putrefaction, active decay and skeletonization. The stages of decomposition described below occur in conventional situations where a complete body is deposited on the surface of a terrestrial natural environment and left to decompose without interference. However, when a dead body is buried, placed underwater or concealed in an artificial environment (e.g. a suitcase), or in an enclosed space (e.g. a vehicle); when modifications have been made to the remains (e.g. mutilation or removal of body parts, etc.); when the body is exposed to extreme environments, such as a desert or frozen landscape; when there is a large interference by macroscavengers who will consume the remains partially or in their entirety, the typical processes of decomposition described below will be altered.

During the fresh stage of decomposition, the body progresses through a series of phenomena known as the mortis triad (11). Rigor mortis involves the stiffening of all

muscles throughout the body, it generally occurs a few hours after death and can persist for up to a few days. Algor mortis is the gradual cooling of the body until it reaches the temperature of the surrounding environment. Livor mortis occurs because of the cessation of blood circulation, leading to red blood cells (RBCs) pooling in the lowest body portions due to gravity. This can lead to an uneven distribution of skin colour across the body. The lowest portions initially appear red, before fading to purple as the blood cells become deoxygenated, whilst the higher portions, or those under pressure, appear white (12). During the second stage, autolysis, the body's cells begin to lyse and break apart, releasing digestive enzymes into the body cavities, as they are no longer regulated by metabolism. This leads to a breakdown of the body's structures from the inside out, as the enzymes and bacteria are left to carry out their destructive functions. The third stage of putrefaction comes as a direct result of this internal destruction, leading to the release of fluids and gases as a by-product of bacterial activity, and manifesting as bloating. This is also typically the stage where insects and other organisms will begin to proliferate and feed on the decomposing soft tissues. The fourth stage of active decay is the result of rapid soft tissue desiccation due to a wide range of variables, including scavenging activity, insect activity, and environmental conditions. Once all of the soft tissue has been removed, the body has progressed into the final stage known as skeletonization (11, 12).

1.4 The taphonomic process in the forensic context

The modifications that occur to an organism after death, as the organic components pass from the biosphere into the lithosphere, are studied in the branch of science known as taphonomy (8, 13). Interest in taphonomy was first gained in this area from geologists and

palaeontologists, who studied fossil preservation (13). Taphonomy now applies to processes altering both modern bone and ancient remains, often overlapping with other disciplines, such as anthropology and palaeontology, in terms of analytical techniques and interpretations of findings (13). Forensic taphonomy, in particular, is a sub-discipline of forensic anthropology that deals with the post-mortem modifications on human remains and any associated evidence, such as clothing, in relation to well-recognised taphonomic agents, such as animals, bacteria, plants, temperature, humidity and chemical alterations (10). These taphonomic agents vary greatly across different natural or artificial environments, from subaerial exposure to burials or submerged conditions.

Understanding the different taphonomic agents common to each depositional environment allows for a more detailed analysis and interpretation of the processes that occurred at the time of death (perimortem) and after death (post-mortem). As the strongest substances in the human body, biomineralized tissues, are often all that is left when all other tissues have decomposed (13). Therefore, determining which processes affected these tissues after death can aid forensic investigators in estimating the circumstances surrounding death, as well as the time since death, also known as the post-mortem interval (PMI). These investigations add clues to the identification of individual/s, which is the starting point of any forensic investigation on human skeletal remains.

1.5 Aim of this review

The aim of this review is the gathering of present information on the taphonomic processes of biomineralized tissues (bones and teeth) from a variety of natural depositional environments (including burial, subaerial exposure, aquatic environments, burnt and

frozen remains). Whilst there are incidences of remains being recovered from a variety of artificial environments such as suitcases, freezers, vehicles, etc., this review will focus exclusively on natural and simple depositional environments; this involves environments in which human remains have been directly deposited without being concealed in anything else prior to deposition. Due to the variability of artificial environments and the lack of current research surrounding the taphonomic processes that occur, only remains deposited directly into natural environments will be considered in this review. This information may be used to assist the forensic community in the reconstruction of the post-mortem history of bones and teeth, which adds clues to forensic investigations.

2. TAPHONOMY IN BURIAL ENVIRONMENTS

Burial environments involve the disposal of the deceased individual through placement of their remains into a grave, either a clandestine hole or pit, or a formal burial in a coffin. This process is also referred to as interment, or inhumation, indicating that the remains are placed at a subterranean level (14). The factors that affect the taphonomic changes in such environments are a combination of the way the bodies have been buried (i.e., type of coffin, if any), geological/physical/chemical characteristics of the soil, and micro/macro-organisms present in the soil (bioerosion). Whilst this review focuses solely on single burials, it is worth mentioning briefly the effects seen in mass burials. When several bodies are deposited into a grave simultaneously, the rate of decomposition is greater at the periphery than the centre, due to increased surface area in contact with the soil and its encompassing taphonomic agents. This 'feather-edge' effect can result in varying taphonomic alterations seen

throughout the remains, which may make interpretation and analysis of the taphonomic history difficult in comparison to single burials (8, 15).

2.1 Outdoor/clandestine graves

2.1.1 Decomposition

Decomposition within a burial environment depends on many variables, including but not limited to burial depth, soil composition and pH, temperature, moisture, bioavailable oxygen, the presence, or absence of a coffin and the coffin's composition. As the remains are buried beneath the soil, they are no longer easily accessible to insect and vertebrate scavengers that typically inhabit surface remains, and instead rely on the microbial community (9). These microorganisms are not so effective as other scavengers, and their limited action, alongside a decrease in temperature and modifications linked to varying soil moisture and pH, can considerably slow the decomposition process, that can take up to eight times longer than that of remains deposited on the surface (16).

2.1.2 Bioerosion

Bacteria

The physiological mechanisms that normally contain the action of enteric bacteria cease their functioning immediately after death. This results in bacteria transmigrating from the gastrointestinal system to all body systems. The body is then used as a feeding ground for the growth of these destructive microorganisms (9). Once the soft tissues

have been removed, specific classes of saprophytic bacteria secrete enzymes known as collagenases, which break down bone collagen for bacterial feeding. This results in the formation of penetrating tunnels and foci within the bone, as shown in Figure 2.1 (17, 18). Bacterial collagenases function at an optimal pH of 7.3-7.4, and are therefore more active within neutral soil environments (9, 17). As the structural integrity of collagen is lost, the thinner parts of the bone start to disintegrate in what is known as cortical exfoliation. The presence of bacteria can also lead to bone discolouration, typically seen as localised black, red, pink, or purple patches across the bone (18, 19).

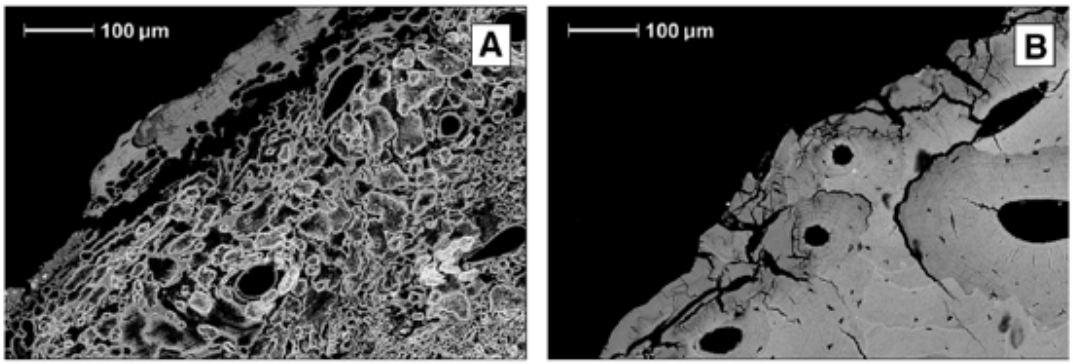

Figure 2.1. A: BSEM image of human bone from a free-draining area showing extensive tunnelling from bacteria. Note that the periosteal bone is characterised by empty pores ~10um in diameter, whereas the outer ~50um that was in contact with the soil is un-tunnelled. B: BSEM image of bone from a waterlogged area, showing an absence of bacterial tunnelling, but with considerable demineralisation and cracking of the periosteal bone. From (20).

Fungi

Fungi are organized in long strings, known as hyphae, that can attach to a variety of substrates to feed and grow. They often grow in large masses, increasing the damage to the substrate (15). Fungi are saprophytic like collagenases-secreting bacteria, and secrete acids to decompose the cortical layer of bone as they extract the desired nutrients (17, 18). Over time, this acid attack can lead to cortical exfoliation of bone, which presents a roughened, pitted surface in localised patches, not unlike the effects

of acidic soil corrosion (15). The fungal hyphae can also penetrate through bone, resulting in the formation of characteristic tunnels and foci (17). Fungi favour more acidic soils, and will be inhibited in high moisture or waterlogged soils, as the anoxic conditions are not compatible with their aerobic metabolism (17).

Root etching

The nitrogen and phosphate content of bone is a highly concentrated source of nutrients for plants. As plant roots seek out nutrients, they may grow in and around bones, leaving behind characteristic damage (15). The roots of plants secrete several chemical compounds, including humic, malic, and citric acids, that will dissolve any areas of bone upon physical contact. Plants secrete these compounds for a variety of reasons, including mineral uptake and deterring herbivorous activity (15, 21). The effect of these secretions on bone is to remove portions of the cortical layer, leaving behind a series of thin, shallow lines that meander across the surface in an irregular pattern. These lines typically have a U-shaped cross-section and can often be mistaken for vascular grooves or tool marks (21). Other forms of damage caused by plants can occur when the roots growing through a bone thicken to the point where they exceed the available space. This expansion is facilitated by trapped water within the porous bone structure, and cause cracking from the inside (15).

Insects

The role that insects play during the process of decomposition is widely known, however less extensive research has been done surrounding insects that colonise and destroy biomineralized tissues, once all the soft tissue has been removed. These insects are not so numerous as microorganisms such as bacteria, but are a lot more efficient in terms of tissue degradation (9). There are at least three orders of insects that are known to alter bone: *Isoptera* (termites), *Coleoptera* (beetles), and *Hymenoptera* (bees, wasps, ants) (9). Subterranean termites are the most destructive insects worldwide, attacking fleshed, dry, and weathered bone and leaving behind a plethora of destruction. Termite damage can appear in a range of forms, including pits, surface etching, star-shaped marks, discolouration of the cortical surface, and bore holes with an average diameter of 3mm (18). Other insects such as cockroaches, ants, and beetles may produce striations and perforations through bone, similar in size to those of termites. Some beetles will also destroy and remove bone to create pupation chambers, which appear flask or bulb-shaped in cross-section and often larger than termite bore holes (18).

2.1.3 Soil characteristics

Soil composition

The type of soil where a body is deposited can influence the number and variety of taphonomic agents causing destruction. Soils with a fine composition are often better at preserving remains, as the small particles will retain higher moisture levels, restricting the proliferation of aerobic microorganisms, and lessening the risk of external damage of bones and teeth through sediment abrasion. Soils with larger particles often have

lower moisture levels and better air flow, allowing for faster desiccation of soft tissues, and leading to higher incidences of damage to the cortical surfaces of bones, as the larger particles act as abrasive agents (9, 18). The effects of sediment abrasion are more likely to be observed after advanced decomposition, when the majority of soft tissues have been removed (18). Various processes, such as bioturbation, geological events, and freeze-thaw cycles can allow for the contact of sedimentary particles against bone (21). This movement can result in the formation of striations along the cortical surface and can also abrade or polish the bone. Under low magnification, such striations appear shallow and commonly intersect with each other. Striae often contain discontinuous and irregularly spaced micro-striations due to the imperfections within sedimentary particles. This is in stark contrast to striations from blades or other sharp instruments, which produce continuous and even micro-striations (18). The soil composition can also impart various forms of discolouration on bones, due to the porous structure and initially pale colouration. Some forms of staining originate from mineral deposits within the soil, such as copper (Cu) and iron (Fe). Tannins, chemical compounds naturally present in most soils, can lead to a dark brown discolouration of bones, and highly oxidised red clay soils can produce a reddish staining. Bones presenting pink or mauve staining have often been colonised by moulds (15).

Soil pH

The pH of the soil where remains are buried plays a role in both the rate of decomposition and in the potential preservation of biomineralized tissues. Neutral and alkaline soils have demonstrated to favour bone preservation, due to the very low

extent of destructive effects when compared to acidic soils, and the relative insolubility of BA (a major structural component of bone) at mid to high pH levels (9, 17). Some damage will still occur at neutral and alkaline pH levels, including discolouration from bacterial species that thrive in such conditions, however the damage is less apparent (22). Acidic soils cause the greatest damage to bones, not only because fungi species thrive in these conditions, but also because of the effects of acidic soil corrosion on the bone structure. At pH levels <5, the demineralisation of the BA component of bone is promoted, leading to a loss of structural integrity (15, 22). Damage is most apparent on weaker structures, such as trabecular bone and the epiphyses of long bones, which can depict a scooped, irregular surface due to loss of BA (15). In extreme cases, remains can become preserved or somewhat mummified if the right conditions are met. Bogs are wetlands characterised by anaerobic, highly acidic waterlogged soils with a high concentration of tannins from decomposing plant materials. These conditions often exclude scavenging activity from local wildlife, further promoting the preservation of soft tissues. This combination of factors results in the tanning of skin into a leather-like substance, whilst bones become demineralized. Bog bodies can remain in this state of preservation for hundreds of years (15).

Soil depth

The depth of burial of interred remains directly influences the rate of decomposition, alongside other taphonomic agents, such as temperature, moisture, access to scavengers and soil organisms, and bioavailable oxygen (9, 16). Shallow burials will decompose faster as they are exposed to higher temperatures, higher humidity,

increased scavenger activity, and are more likely to be affected by organisms like fungi and bacteria. Deeper burials result in lower temperatures and humidity, and are more restrictive to scavengers and other organism activity (22). Historical burials have been found at depths as shallow as 2ft and as deep as 5.5ft, whereas modern burials tend to be anywhere from 1.5-12ft, depending on cemetery requirements (15). Clandestine graves, dug into the ground to conceal the bodies of victims of criminal activity, are usually shallow (23).

Soil moisture

The moisture content of the soil where remains are deposited can promote or prevent the breakdown of tissues. Low moisture soils with good aeration and bioavailable oxygen, such as sandy soils, promote desiccation, whilst waterlogged soils or those with high moisture levels, such as clay soils, often reduce the rate of desiccation leading to better preserved tissues (9, 17, 24). The higher moisture content reduces the activity of aerobic-dependent organisms, such as certain bacteria and fungi, and allows for the creation of a buffered environment surrounding the tissues due to a lack of percolation (17). High moisture, anaerobic environments are also ideal for the formation of adipocere, which is often referred to as 'grave wax'. Adipocere forms through the hydrolysis of triglycerides into insoluble hydroxy and saturated fats via anaerobic bacterial action. The presence of adipocere often acts as a protective shell, restricting access to other taphonomic agents, such as scavengers or oxidative mechanisms of degradation (15, 25).

2.2 CEMETERY BURIALS

Cemeteries comprise many types of lawful burials depending on geographic location and environmental conditions, age of the cemetery and whether it is still in operation, and the types of people permitted to be buried within the grounds (rich, poor, religious or not). Factors such as wealth and age of the cemetery may affect the type of coffin used in the burial, from cheaper wood varieties to more expensive metal variations; these factors can directly impact the rate of decomposition and types of taphonomic alterations observed. Similarly, the geographic location of the cemetery, and thus the surrounding environment, can affect the degree and type of taphonomic alterations seen on remains. Cemeteries located in desert climates are more likely to result in the inhibition of decomposition (mummification) due to low humidity levels and extreme temperatures, whereas cemeteries located within tropical climates will exhibit accelerated decomposition rates in response to higher humidity levels and a larger abundance of available scavengers (26, 27).

Due to the current lack of research surrounding decomposition and taphonomy for cemetery burials within extreme environments, this review will focus only on burials that occur in typical cemetery settings, located in temperate environments, where temperature and humidity levels are more stable.

Decomposition of human remains in a damaged, or degraded, coffin proceeds at a similar rate to that of non-coffin burials (16). The airtight environment of a coffin or tomb prevents taphonomic alteration by insect and vertebrate scavengers, whilst the effects of high temperatures and humidity, that speed up the rate of decomposition in exposed remains, are reduced. Coffins limit the exposure of the remains to the soil

environment until the collapse or degradation of the coffin structure. Once exposed to the soil environment, remains are affected by the same taphonomic agents as non-coffin burials, including soil pH levels, moisture and invading soil organisms, such as fungi and microbes (16).

2.2.1 Coffin composition

The composition of a coffin determines the ensuing rate of decomposition of the body, as some coffin materials are more durable and less likely to decay as quickly as others (15). Wooden coffins were typically constructed using pine, due to its availability and low cost, though any hard or softwood could be used (15, 28). Skeletal elements recovered from wooden coffin burials often exhibit a range of staining due to several factors. The coffin wood itself is an excellent source of tannins, which are found in trunks and other plant material, and can lead to a pattern of uniform chocolate-brown staining of bones when these tannins leach out of the wood due to its breakdown over time, or through the pooling of water in the coffin which has seeped through the wooden walls (28). Lighter species of wood tend to have lower concentrations of tannins, therefore resulting in reduced staining of skeletal elements (28). This type of staining is typically more uniform than that of bones on the ground surface, where staining appears randomly distributed and more varied in colour. Another form of staining occurs when metal ions come into contact with skeletal elements, such as through contact with coffin hardware, or when these materials are leached into groundwater that seeps into the coffin. Metals such as copper (Cu) and iron (Fe) can

produce green, red, or dark metallic colours on the bones. This can be an indicator to forensic investigators of a coffin burial, when the coffin has completely degraded (28).

Metal coffins (e.g., iron, zinc, lead) present another unique burial environment, as they are more durable than wooden coffins, and more suited to preventing the degradative effects of burial taphonomic agents upon skeletal elements (15, 29). The lids of metal coffins are more effectively sealed than those of wooden coffins, as a sealant consisting of a mixture of ground lead and oil is used, which creates an internal anoxic environment, preventing any air exchange with the external environment. Some metal coffins also contain a viewing window which is used during the funeral, to view the body whilst keeping the coffin lid sealed. The inclusion of a window potentially lessens the structural integrity of the coffin walls, leading to increased risk of failure once interred, as the weight of the soil presses against the walls. Skeletal remains found within metal coffins are typically stained a dark or black colour, due to the leaching of mineral oxides from the coffin walls (15). Skeletal remains recovered from above-ground crypts have been shown to exhibit a uniform orange-brown staining, believed to be the result of a lack of groundwater to transport other minerals to and from the remains, and the presence of embalming chemicals applied before burial (15, 28).

2.2.2 Coffin wear and warping

Coffin wear is the localised destruction of a bone due to constant and prolonged contact with the coffin floor, basically a form of friction. It occurs after decomposition has progressed to the point where most soft tissue has been removed (15, 16, 28). Coffin wear presents as concentrated cortical erosion of posterior bony elements, often with

a flattened or sheared appearance (Figure 2.2). The most common bones affected by coffin wear include the posterior elements of the cranium, calcaneus, pelvis, scapulae, and vertebral spines, that is, elements that remain stationary for long periods of time, as opposed to those that may shift or rotate as decomposition progresses. This pattern of destruction is exacerbated by the presence of pooling water within the coffin, which tends to be more acidic in nature due to the low pH of the decomposition fluids (15, 28).

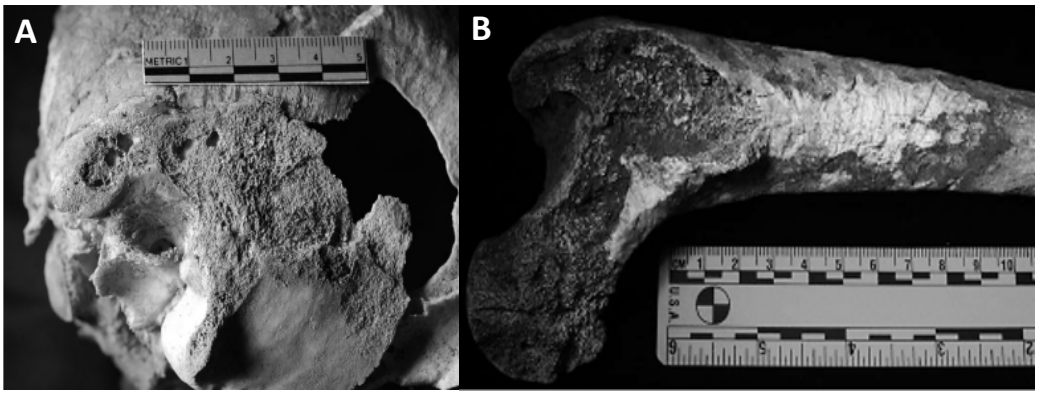

Figure 2.2. A: coffin wear to the right temporal of a human skull. B: Coffin wear to a proximal right femur. Note the erosion of the bone into a flat, sheared appearance. From (28).

Warping of skeletal elements from coffin environments may occur through similar mechanisms as coffin wear. The pooling of acidic water at the base of the coffin contributes to the degradation of the bone matrix, making it more prone to deformation and damage. Compression also likely contributes to skeletal warping as the coffin environment becomes compromised by soil, due to weakening of the walls over time. The cranium is particularly affected by warping due to its weaker bone structure relative to size and the lesser amount of soft tissue needed to be removed before exposure of the bone (15, 28).

2.2.3 Teeth (coffin environments)

There is currently a lack of research surrounding the diagenesis of teeth in coffin environments, though one study of remains from a Bronze Age burial demonstrated excellent preservation of the enamel, along with other proteinaceous material such as fingernails and hair, whilst all other skeletal material had disintegrated. Kendall et al., 2018 (20) speculated that this differential preservation was due to the leaching of tannins from the oak coffin, in combination with the waterlogged and anoxic coffin environment (20).

3. TAPHONOMY IN SUBAERIAL ENVIRONMENTS

The deposition of remains at the ground surface, exposed to the natural elements without the protection of a burial, characterises subaerial environments. In that condition, remains gradually undergo decomposition, separation and destruction of both organic and inorganic components (30, 31).

Subaerial environments can encompass a variety of environments, from temperate regions to desert, tropical and frozen landscapes. The microenvironment where the remains are deposited has a great influence on the body's decomposition rates, with more sheltered, temperate climates shown to decrease decomposition activities, whilst open, moist and hot environments with abundant sun exposure will lead to rapid decomposition (26, 27). The effects that such extreme conditions have on deposited remains, however, such as largely increased or decreased temperatures, and high humidity levels, is not widely researched. It could be posited that such extreme temperature variations may inhibit decomposition

entirely, resulting in the mummification of remains and an entirely different taphonomic history to remains deposited into a more typical, temperature subaerial environment. The decomposition of remains in a temperate subaerial environment is often more rapid than any other natural depositional environment, e.g., aquatic or burial, as they are exposed to a larger number of variables. Insects and scavengers can easily access the remains, and the rate of desiccation is often increased due to higher humidity levels. The process of subaerial decomposition can ultimately be seen as the most natural method of recycling the body's chemical components back into the environment (31). This review will focus largely on subaerial environments in temperate climates, which have been more extensively researched, with only brief mention of the effects seen in other climates. Whilst subaerial environments may become secondary depositional environments at some point in the taphonomic history of the remains, e.g., when a bone washes up on the shoreline and is taken into the forest by a scavenger, only research pertaining to the taphonomic alterations seen on remains where a subaerial environment was their primary location of deposition will be considered below.

3.1 Subaerial weathering

Subaerial weathering is a taphonomic process whereby the biomineralized tissues decay and break down over time. This is due to the prolonged exposure to various taphonomic agents that work mechanically, biologically, and chemically to separate and destroy their organic and inorganic components (27, 30, 32). The weathering begins once the decomposition process has removed most, or all, soft tissues (8). Bone undergoes cortical exfoliation, flaking and cracking of the surface layers, bleaching, loss of moisture and total

disintegration, as the bone minerals and collagen are progressively lost, as shown in Figure 3.1 (27, 32, 33). The degree of deterioration is the basis for determining the weathering stage of bones and teeth, as defined in table 3.1. The rate of weathering is largely affected by the climate and the depositional microenvironment of the remains, which will still progress through each stage sequentially regardless of location (26, 33). The microenvironment will dictate the extent of vegetation coverage, the amount of direct sun exposure, the temperature and humidity fluctuations as well as the exposure to freeze-thaw and wet-dry cycles (26, 32). Weathered bones become more susceptible to other taphonomic agents, such as rodents, who preferentially gnaw on dry, weathered bone to both obtain nutrients and wear down their teeth (33).

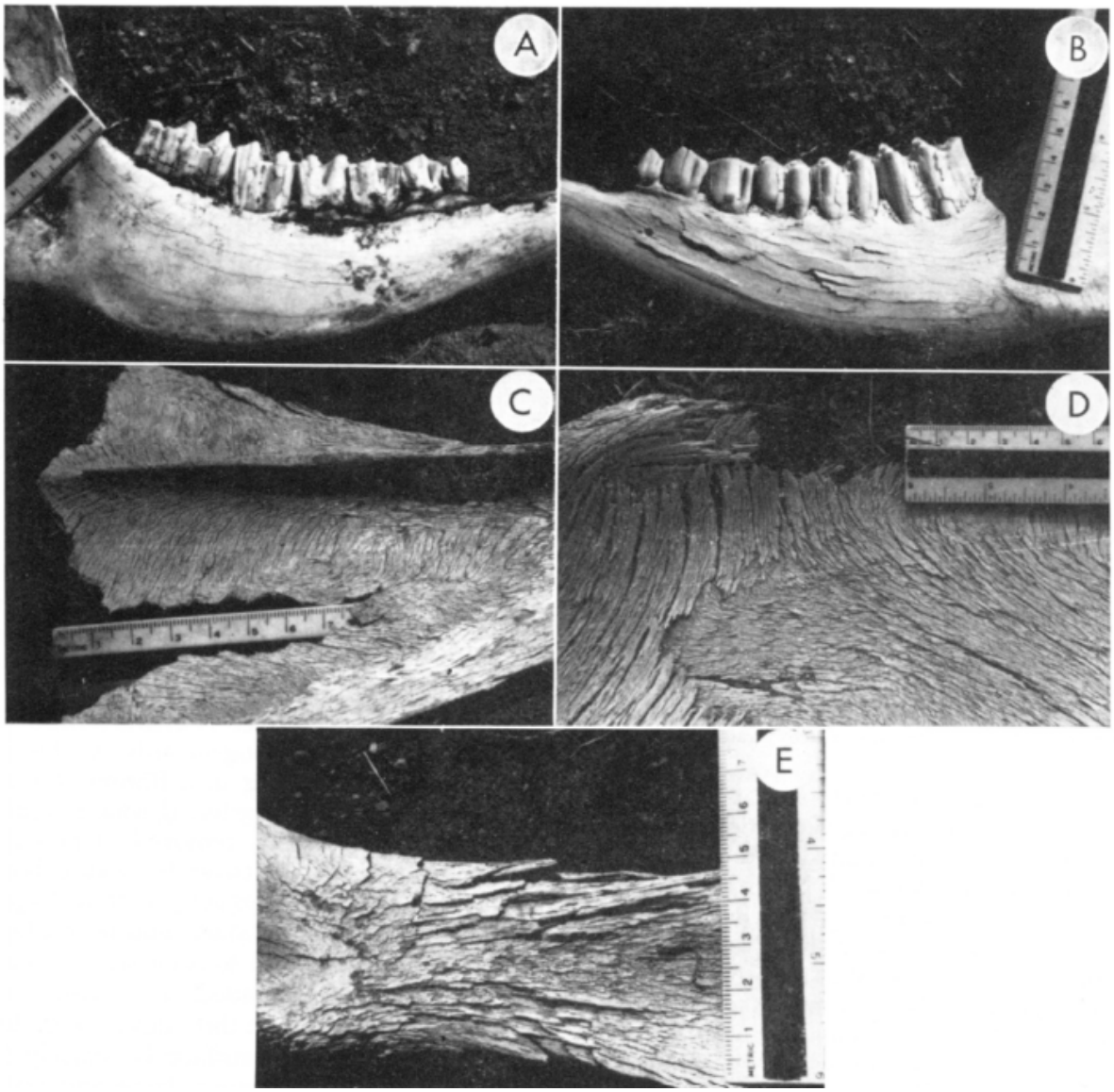

Figure 3.1. A bone assemblage representing weathering stage 1-5. A: a cow mandible showing parallel cracking consistent with stage 1. B: a cow mandible showing flaking of outer bone layers, consistent with stage 2. C: Bovid scapula showing fibrous, rough texture and remnants of surface bone, stage 3. D: Bovid scapula showing deep cracking, coarse, layered fibre structure, stage 4. E: scapula blade showing final stages of deep cracking and splitting, stage 5. Note: a 15cm scale is shown in all photos. From (31).

Table 3.1. Weathering stages and their definitions based on a study of animal remains across several varied depositional environments

Weathering Stage	Definition
0	Bone surface shows no sign of cracking or flaking due to weathering. Usually bone is still greasy, marrow cavities contain tissue, skin and muscle/ligament may cover part or all the bone surface.
1	Bone shows cracking, normally parallel to the fibre structure (e.g., longitudinal in long bones). Articular surfaces may show mosaic cracking of covering tissue as well as in the bone itself. Fat, skin, and other tissue may or may not be present.
2	Outermost concentric thin layers of bone show flaking, usually associated with cracks, in that the bone edges along the cracks tend to separate and flake first. Long thin flakes, with one or more sides still attached to the bone, are common in the initial part of Stage 2. Deeper and more extensive flaking follows, until most of the outermost bone is gone. Crack edges are usually angular in cross-section. Remnants of ligaments, cartilage, and skin may be present.
3	Bone surface is characterised by patches of rough, homogenously weathered compact bone, resulting in a fibrous texture. In these patches, all the external, concentrically layered bone has been removed. Gradually the patches extend to cover the entire bone surface. Weathering does not penetrate deeper than 1.0-1.5mm at this stage.
4	The bone surface is coarsely fibrous and rough in texture; large and small splinters occur and may be loose enough to fall away from the bone when it is moved. Weathering penetrated inner cavities. Cracks are open and have splintered or rounded edges.
5	Bone is falling apart in situ, with large splinters lying around what remains of the whole, which is fragile and easily broken by moving. Original bone shape may be difficult to determine. Trabecular bone is usually exposed, when present, and may outlast all traces of the former more compact, outer parts of the bone.

Adapted from (31)

To determine the stage of a bone's weathering, various criteria need to be met, though often weathering is not a uniform process across the entire surface of any bone. Due to the uneven distribution, the most advanced weathering stage covering more than 1cm^2 of the bone's surface is recorded as the weathering stage for the entire bone. Typically, long bones, vertebrae, pelves or ribs are used, as these bones have a greater flat surface area

on which to observe weathering processes. For a weathering stage to be confirmed, more than one observer must agree on the determination (31).

When assessing the weathering stage of a complete set of remains rather than an individual component, it is recommended to examine several different bones (e.g., a femur, vertebrae, and a rib) to assess the most advanced weathering stage. Generally, weathering stages are only applied to mammals of mass greater than 5 kg, as there is not enough research completed on weathering of biomineralized tissues in smaller animals (31).

As rates of weathering are linked to time since death, they can cautiously be used as a proxy for the estimation of the PMI in forensic cases, however the rate of decomposition of soft tissues will need to be considered, as bone weathering will not begin until soft tissues persist (30).

3.2 UV exposure

The prolonged exposure of bones to UV light and heat from the sun is the primary cause of weathering in subaerial environments. Two processes occur almost simultaneously after bone becomes exposed: bleaching and cracking. Bleaching begins early in the weathering process, as the UV rays break down the bonds between molecules within the bone matrix, resulting in photodegradation, or the loss of colour (33, 34). One study demonstrated that bleaching occurred more rapidly in summer than in winter, and that bones deposited in shady or sheltered areas did not bleach at all (34). Severely bleached bone can be mistaken for prolonged thermal alteration, such as calcined bone. However, calcined bone presents a much smoother surface in comparison to weathered bone (33).

The cracking of bone begins as moisture evaporates, and the natural organic and mineral contents are gradually broken down (8, 30). The loss of moisture results in the shrinkage of the uppermost layer of cortical bone, placing tension on the layers underneath. Cracking will occur to relieve the tension, and prolonged exposure will lead to the removal of the top layer in flakes, known as delamination, with the process continuing for each layer until the bone has completely disintegrated (8, 33). Uneven drying of the bone can also result in the formation of longitudinal cracks or warping if one side of the bone dries faster. Weathering typically produces cracks that run parallel to osteon orientations, following the weaker planes of the bone. Irregularly shaped bones such as vertebrae show irregular cracking in multiple directions as the osteons are aligned along the higher stress points (33). The degree of sun exposure will determine the rate of crack propagation and bleaching on exposed bones, with some remains showing little degradation after 30 years, while others may be severely eroded in as little as six years (27).

3.3 Temperature and humidity

Fluctuating temperature and humidity levels, which typically occur diurnally, will lead to more rapid bone degradation, especially in open environments. Sheltered environments tend to delay degradation of bone tissue due to the increased vegetation cover, and more constant temperature and humidity (27, 33). Rapid changes in the levels of temperature and moisture cause the expansion and the retraction of bone, which can lead to cracking as the stress overcomes its tensile strength (33). The upper surfaces of bones will be more greatly affected by these changes, as they are directly exposed to the sun and any occurring

precipitation. The lower portions, in contact with the soil surface, are protected from most environmental fluctuations, and often exhibit considerably slower weathering (31).

3.4 Freeze-thaw and wet-dry cycles

3.4.1 Freeze-thaw cycles

Extreme fluctuations in temperatures, such as those below freezing (0°C/32°F), to more moderate temperatures in the range of 20-25°C (68-77°F) may lead to damage in the form of cracking, flaking, and warping, with such effects exacerbated when moisture is present within the bone, due to the expansion water molecules undergo upon freezing, which exert a large destructive force upon the surrounding structures (33).

Bone deposited in environments subjected to frequent freeze-thaw cycles tend to degrade faster than in those with more constant temperatures, such as subtropical or tundra regions. Water from the surrounding environment will seep into pre-existing bone cracks and any available pore spaces (33). This generally involves the trabecular bone and any damage already apparent in the bone. However, cortical bone also contains pore spaces in as much as 12% of the bone volume, so it can also be affected by damage connected to the freeze-thaw cycle (30). As water freezes, it expands due to the formation of ice crystals, which can exert a 30,000 lb force per in^2, exceeding the maximum tensile strength of bone (30, 33). This force can lead to the formation of new cracks and will often expand existing cracks wider and deeper into the bone. As the ice thaws, more room is available for water to occupy, which may then re-freeze, continuing the cycle of destruction. Prolonged exposure of bones to environments

characterised by frequent freeze-thaw cycles can result in significant damage and bone degradation (33).

3.4.2 Wet-dry cycles

When bone is exposed to moisture in the form of precipitation or contact with groundwater in the soil, the tissues will partially rehydrate, until they once again become dried out by daily UV exposure. The amount of moisture and UV exposure remains are exposed to depends solely on the microenvironment of the depositional location (33). Repeated wet-dry cycles over time can generate damage in the form of cracking, flaking, and warping.

Bone tissue exposed to the external environment will lose moisture through several mechanisms. Primarily, soft tissue will be lost through decomposition, followed by a loss of internal water through evaporation. Finally, bone grease will eventually leach from bones or be consumed by scavengers. The drying of bone will cause shrinkage, resulting in cracking and flaking of the cortical surface, as successive layers are eventually removed (33). Studies analysing the effects of repeated wet-dry cycles on bone tissue have observed the formation of deep longitudinal cracks penetrating as far as the medullary (marrow) cavity in bovid bones (35), with another study concluding that long bones were more affected than short bones, and often showed high degrees of cortical exfoliation (36). Bones may regain moisture through precipitations or from the soil and groundwater, which will cause expansion of the tissues as they rehydrate (30, 33). The degree of moisture available for reuptake by bone tissue is dependent on the depositional environment, as tropical and rainforest areas will tend to produce

more rainfall and be more sheltered from heat and sunlight than arid, desert environments where rainfall may be sparse (30).

When bones uptake moisture from the soil, soluble/exchangeable minerals from the groundwater can infiltrate the bone structure and result in the formation of authigenic crystals as the water evaporates (33, 37). These crystals can alter the physical and microscopic structure of the bone, weakening the bone matrix through recrystallisation of the BA component, and resulting in a less homogeneous overall composition (Figure 3.2)(33). The filtration and evaporation of pore waters through bone can also alter the trace element composition (38). Chemical and rare earth elements (REEs) increase in concentration the longer bones are exposed and infiltrated by pore waters. These elements are easily taken up into the bone structure as the adsorption coefficient between bone apatite and water is very high. Eventually the bones will reflect a trace element composition identical to that of the pore water, which provides an excellent geographical marker of their depositional location (37). This may prove beneficial to forensic investigations where remains are suspected to have been deposited in more than one location in the post-mortem period.

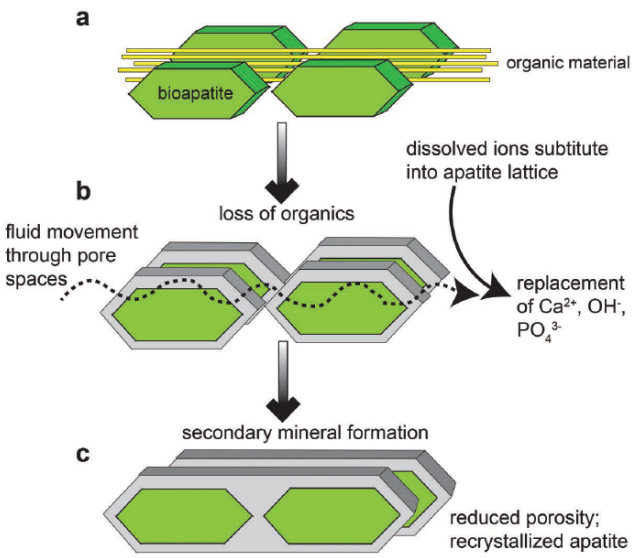

Figure 3.2. A: bioapatite consists of interlayered mineral and organic phases. B: The degradation of collagen opens pore spaces to the movements of fluids carrying dissolved ions. C: Substitution of elements in the bioapatite lattice results in the formation of secondary mineral phases, with reduced porosity, and increased crystallite size. From (39).

3.5 Bioerosion

3.5.1 Algae and fungi

Algae are eukaryotic organisms that obtain their energy via photosynthesis. The conditions needed by algae to grow, e.g., sunlight, moisture and nutrients, are the same factors that favour the weathering of bone in subaerial (exposed) environments (33). Algae growth is common on exposed bones, in particular on the surface that is not in contact with the soil. Evidence of algal growth on bone can be seen through the formation of microtunnels, which appear more superficial and homogenous than damage from other bone-altering organisms. Algae typically penetrate bone using the Haversian canals, as they don't have a mechanism of dissolving bone, like other species such as fungi (40).

Fungi are also eukaryotic organisms, utilising the same conditions as algae for growth. Fungal damage is well-researched, especially on buried bones. However, fungi have been suspected to affect the surface of deposited bone, producing microscopic focal damage known as Wedl-tunnelling (33, 40).

3.5.2 Moss

Mosses are small, non-vascular plants that tend to grow in groups, or patches, across several substrates. They derive their energy from the process of photosynthesis, requiring sunlight and moisture to survive. Moss can often use bone as a growth substrate, utilising microanatomical structures, such as Haversian canals, to intrude further inside (33). When mosses are removed from bone, several features can be identified, including surface staining , microtunnelling, small pitting, and edge rounding of surfaces. Mosses can sometimes break thin bones, such as the trabecular bone structures, or even immature cortical bone, due to their expansion during growth (40).

3.5.3 Lichen

Lichens are symbiotic organisms consisting of both a fungi and plant form (a cyanobacteria or algae). The differing properties of lichens requires them to rely on their counterparts to survive. The cyanobacteria or algae allow the fungi to obtain nutrients through photosynthesis, and the fungus, in turn, provides an ideal sheltered and moist environment to the cyanobacteria or algae (33). Lichen can grow on several substrates, and are numerous in appearance, only requiring sunlight and moisture to propagate. Lichen's effects on bone are similar to that of algae and moss, with frequent

shallow microtunnelling observed, alongside holes, fissures and cracks within the cortical bone surface (40).

3.6 Soil acidity corrosion

In many cases, bones will appear more weathered on the upper surface than on the lower. This is due to increased exposure to UV light, and larger fluctuations in temperature and humidity at the upper surface, compared to the stable soil environment at the lower surface (31). This difference is more apparent when the pH of the underlying soil is very low (acidic). The bone surface in contact with the soil can also be influenced by other taphonomic agents such as bacteria, fungal and plant root activity, and subterranean insect activity (31). Evidence of acidic soil corrosion of bone appears both macroscopically and histologically, and prolonged contact with the soil will increase these effects. The surface of the bone may show scooping out of the articular ends, with cortical layer degradation due to the corrosive activity of the soil. The overall appearance has been compared to that of bone digested by gastric stomach acids when examined using scanning electron microscopy (SEM)(40). Histologically, acidic soil corrosion appears as thin microtunnelling with reduced bone density, and degradation of the cortical layer to the point where osteons and interstitial lamellae are exposed (40).

3.7 Teeth

With the exception of some experimental studies, relatively little information is available on the taphonomic alterations affecting teeth in a subaerial depositional environment,. One study assessed the impact of summer temperatures on the teeth of *Sus scrofa,*

demonstrating that cracks will begin in the pulp cavity before proceeding outwards towards the dentin-enamel junction, where the strength of the junction would prevent the crack from spreading further. The overall pattern seen in this study was that the cracks progressed from the inside out, in direct contrast to cracks formed through trauma to the tooth, which travel from the enamel surface inwards (8). Another study looked at several variables affecting teeth in subaerial environments, concluding that whilst prolonged exposure to such conditions can result in the cracking of the teeth, other individual characteristics will also play a role, such as the degree of enamel wear, the stage of eruption and the overall morphology (31).

4. TAPHONOMY OF BURNT TISSUE

Burnt remains have undergone thermal alteration through combustion for varying time durations because of several possible events, such as motor vehicle accidents, plane crashes, house fires and natural disasters. The effects of this form of alteration are have been identified and categorised (41).

4.1 Commercial cremation

Modern commercial cremation reduces the deceased's body into ash, and it is proposed as an alternative to a traditional burial. The cremation process is generally completed in two steps, the incineration and the following processing of any remaining bone fragments (42). The remains are first placed into a container, e.g., a simple wooden coffin, which enters feet-first into a cremation oven, called a retort, where the cremation process begins (41, 42). Commercial crematoria operate at temperatures between 800-1000°C for an

average of 2-3 hours, depending on several factors, most notably the size of the deceased individual (41-43). A large proportion of body mass is lost early in the process, due to the breakdown of soft tissues and evaporation of moisture and collagen from the bones. Once the cremation is complete, some large bone fragments may remain, often exhibiting a white or blue-grey colouring typical of calcination, a stage in which no organic material persists (42). After cremation, the remains are collected from the retort into a smaller container and processed to ensure that all the remaining bone fragments and teeth are reduced to ash. This is the stage where any surgical implants/devices or any other metal object (e.g., staples) are removed using a magnet. To pulverise the remains to the desired particulate size, an electrical device called a cremulator is used (41, 42). After processing, the remains are stored for return to the family or for direct inurnment or scattering. Cremation tags are commonly employed within commercial crematoriums to ensure that the remains are identified correctly (42).

4.2 Outdoor/clandestine cremation

4.2.1 Temperature

Physical appearance

Bone subjected to intense heat exposure progresses through four identifiable stages: dehydration, decomposition, inversion, and fusion (43, 44). Dehydration, the first stage, involves the loss of water from bone, both surface water and water contained within the matrix, which results in warping and fracturing (44). There are several common fracture patterns observed in burnt bones, of which longitudinal fractures are the most common and occur in long bones. Once the moisture has evaporated, bone will begin

to shrink rapidly, leading to fractures that run the length of the shaft, often parallel with histological structures such as Haversian canals. These fractures can also manifest helically, spiralling longitudinally down the axis of long bones (41, 45). The second most commonly occurring heat-related bone fractures are transverse, manifesting in the opposite orientation to longitudinal fractures. Transverse fractures often appear alongside longitudinal fractures, transecting the Haversian canals. This is due to the rapid shrinking of the bone tissues in increments as the soft tissues recede. Patina fractures are likened to the cracking seen in old chinaware and are most commonly observed superficially on flat postcranial bones, such as ribs. Patina fractures are suggested to result from large areas being heated homogenously and shrinking simultaneously. Splintering and delamination of the cortical surface is also common during the dehydration stage, with small layers peeling away. This can also lead to exposure of the trabecular bone at the epiphyses (41, 45).

The decomposition stage follows the dehydration stage, as bone is subjected to prolonged heat exposure. This is where the majority of the organic component of bone, collagen, is removed, resulting in shrinkage and carbonisation (43, 44). As the organic component is lost, elements such as oxygen and hydrogen are liberated, leaving behind carbon and the inorganic component of BA, which appears black (41).

The inversion stage involves the devolatilization of the remaining organic components, resulting in the liberation of carbon as it combines with oxygen molecules to form CO_2 or O_2. With only the inorganic components remaining, bone becomes calcined, appearing white or blue-grey in colour (41, 43, 44). Due to the loss of all organic components, calcined bone is extremely brittle and prone to further physical damage.

Fusion, the last stage, consists of the fusing of inorganic mineral salts into a homogenous unit (41).

These progressive stages roughly follow the increase of the temperature reached during cremation. A summary of the physical, chemical, and biological changes of skeletal tissue associated with each stage is provided in table 4.1.

Colour changes

Bone progresses through distinct colour changes as the duration and the temperature of heat exposure increase. Colours reflect the proportion of organic and inorganic components remaining in bone, or in rare cases, result from a reaction between the bone components and metals present in the depositional environment at the time of burning (43, 46, 47). Copper has been shown to produce a pink discolouration in cremated remains, whilst iron and zinc produce a green and yellow discolouration, respectively (48). Black-coloured bones, which is the typical colouring of carbonised bones, can also be the result of staining by manganese or iron oxides. Analysis of the infrared (IR) spectrums of these compounds can distinguish the difference between carbonised and black stained bones (47). Generally, bones will progress from a translucent white colour to a black colour during carbonisation, to a final white or blue-grey colour once calcination has been reached (43). A summary of the colour changes that occur at varying temperatures is provided in table 4.1.

Histology and crystallinity

Morphological changes in the microstructure of bone and teeth. that occur due to intense and prolonged heat exposure, can be identified at the microscopic level through histology. These microstructures, measured on micron scale, include changes in bone porosity, microcracking, and a gradual reduction or complete disappearance of structures such as osteocyte lacunae, lamellae, Haversian systems, and Volkmann's canals. Studies have demonstrated that with prolonged heat exposure and increasing temperatures, a reduction in the size and the shape of many of these structures is observed, leaving behind a uniformly granular matrix (43, 49, 50).

Crystallinity refers to multiple characteristics of BA crystals within bone and teeth. Fresh bone typically has low crystallinity and small crystal sizes, and the BA component is highly reactive in this form (51). As the thermal alteration progresses, the crystal sizes increase and take on new shapes and a more regular arrangement, and the reactivity of BA decreases (50, 51). This change in crystals structure is also observed in archaeological specimens who have been exposed to diagenetic processes for many years. To measure changes in crystallinity, techniques such as infrared (IR) spectroscopy and X-ray diffraction are commonly utilised (51, 52).

Chemistry

The chemical structure of burnt bones and teeth can also be analysed alongside the physical appearance to provide a more detailed understanding of the degree of thermal alteration that has occurred. When a certain temperature is reached, the crystals will begin to coalesce as fusion and mineral sintering occur, eventually

resulting in the formation of small, rounded granular structures. This process will occur until the complete cremation of bone and teeth, when only ash remains (44, 50, 53). Stable isotope analysis can be performed on bone and teeth tissues to provide information about the history of an individual, including their geographical origins and lifestyle. This technique is particularly beneficial when other methods of identification, including DNA analysis, are not viable. Both light isotopes (C, N) and heavy isotopes (^{18}O, $\delta^{13}C$, $\delta^{15}C$) can be analysed, though results are shown to be reliable only to a temperature of approximately 200°C. This is typically when bone reaches the stage of cremation where it begins to turn brown in colour, but has not yet carbonised (52).

Biological changes

Increased thermal exposure will reduce the amount of collagen in biomineralized tissues and degrade DNA to a point where extraction methods are no longer viable. However, a consensus has not yet been reached in the literature regarding the maximum temperature at which DNA analysis is still possible. Collagen is responsible for the elasticity and structural integrity of bone and makes up most of the organic component. With increasing temperatures, collagen is gradually lost, resulting in the carbonisation of bone, which occurs around 500-600°C (50, 52). The analysis of collagen content is achieved through histology, by measuring birefringence in polarised light or collagen content by specific staining techniques in ordinary light microscopy (54-56).

A decrease in birefringence, or specifically stained areas, in histological slides indicate a reduction in collagen content, which will gradually become absent once all collagen has been depleted (52). DNA analysis is frequently used in forensic investigations to identify

remains, however when they have been exposed to prolonged heat, the integrity of the DNA is compromised to the point where DNA analysis may no longer be an option (52). Some studies were able to successfully extract and amplify readable mitochondrial DNA (mtDNA) from samples that had been subjected to temperatures of up to 700°C, though the quality of results was significantly reduced (57). Whether or not a sample will be able to produce sufficient DNA results can only be assessed through histological and birefringence analysis. If identifiable microanatomical bone features, such as osteons and Haversian canals, are visible on histological images, and birefringence under polarised light is still observable, DNA analysis should be attempted (52).

A summary of the physical, chemical, and biological changes that occur at varying temperatures in burnt bone is provided in table 4.1.

Table 4.1. Physical, chemical, and biological changes to bone associated with increasing thermal alteration

Physical Characterisation				Chemical composition		Biological characterisation			Temperature (°C)
Macroscopic appearance	*Colour*	*Microstructure (histology)*	*Crystallinity*	*Chemistry*	*Isotope analysis*	*DNA*	*Collagen*	*UV-Fluorescence*	
150-300: 1-2% shrinkage observed as moisture and collagen are lost (first major shrinkage stage) <200: dehydration and shrinkage of bone (loss of surface and bound water through evaporation); weight loss <285: irregular bone surface; small, granular asperites, separated from each other by tiny pores and fissures; bone surface remains intact and continuous	Natural off-white/light ivory Yellow Very pale brown Ochraceous Brownish	100: remains of muscular tissue and blood cells; cord-like collagen fibres; Haversian canals deform 200: bar-like arrangement of collagen fibres; longitudinal microfractures in cluster form; small fissures <300: undulating 'glassy' surface with occasional longitudinal fractures in trabecular and cortical bone; clearly determinable osseous cells and remaining organic tissue; disintegration of collagen fibres; trabecular bone structure deforms	200: polyhedral crystalline formation 300: polyhedral crystalline formations in connective tissue matrix 100-300: average crystallite size 170-188Å	-	0: $\delta^{13}C$ (collagen) = -14‰ $\delta^{15}C$ (collagen) = 6‰ $\delta^{13}C$ (carbonate) = -7‰ $\delta^{15}C$ (carbonate) = -9‰ 100: $\delta^{13}C$ (collagen) = -14‰ $\delta^{15}C$ (collagen) = 6‰ $\delta^{13}C$ (carbonate) = -6‰ $\delta^{15}C$ (carbonate) = -9‰ 200: $\delta^{13}C$ (collagen) = -15‰ $\delta^{15}C$ (collagen) = 6‰ $\delta^{13}C$ (carbonate) = -6‰ $\delta^{15}C$ (carbonate) = -8‰ 300: $\delta^{13}C$ (collagen) = -16‰ $\delta^{15}C$ (collagen) = 4‰ $\delta^{13}C$ (carbonate) = -7‰ $\delta^{15}C$ (carbonate) = -11‰	Normal amplification achieved (mtDNA)	0: 11 wt.% 200: 1 wt.% 300: 0.5 wt.%	200: brown 300: black	<300 'Dehydration'
300-400C: 'glassy' layer of char <440: pores and asperites of pervious stage disappear; bone surface becomes glassy and smoother; areas of bone take on vitrified appearance <650: pitted and 'frothy' bone surfaces	Very dark grey brown Brown Dark Grey Black	300: charring temp where most noncarbon elements of the organic components dissociate, leaving only carbon <400: collagen denatures and separates in a cord-like structure, becoming more compact and exhibiting an irregular surface; large areas of bone surface covered with a peeling, bubbly layer of char, with a granular surface underneath 400: lamellae hardly recognisable 500: Overall structure hardly visible, fissures readily apparent 538-816: ~16% shrinkage of osteon diameters observed	400: beginning of cubic-shaped crystalline formations 500: cubic-shaped crystals; onset of linear macromolecular crystal polymer phase 600: cubic crystalline formation in cortical bone gives way to irregular crystalloid structures <600: formation of macromolecular collagen polymer crystals caused by dissociation of HA and thermal destruction of collagen; makes surface appear granular; HA crystals increase in size as organic content decreases <650: formation of new spherical crystals 300-600: average crystallite size 188-256Å	-	400: $\delta^{13}C$ (collagen) = -19‰ $\delta^{15}C$ (collagen) = 1‰ $\delta^{13}C$ (carbonate) = -7‰ $\delta^{15}C$ (carbonate) = -12‰ 500: $\delta^{13}C$ (collagen) = -20‰ $\delta^{15}C$ (collagen) = 2‰ $\delta^{13}C$ (carbonate) = -8‰ $\delta^{15}C$ (carbonate) = -13‰ 600: $\delta^{13}C$ (collagen) = -23‰ $\delta^{15}C$ (collagen) = 1‰ $\delta^{13}C$ (carbonate) = -8‰ $\delta^{15}C$ (carbonate) = -14‰ 500- 600: reduced birefringence as collagen	400: amplification products not successful for each sample (lowered viability) (mtDNA)	400: 0.4 wt.% 500: 0.2 wt.% 600: 0.3 wt.%	400: black (TI) – brown 500: black (TI) – grey	300-600 'Decomposition'

		<600: initial fraction of CO_2 lost due to combustion of organic phase; decomp of structural and secondary carbonate results in loss of second fraction of CO_2; all collagen pyrolyzed (to form carbonate apatite); increasing roughness of trabecular bone			has lost its birefringence in polarised light				
Bone surface becomes highly particulate; rapidly turns frothy 750-800: 1-2% shrinkage observed as carbon content lost (second major shrinkage stage)	Blue-grey White (calcined)	<700: release of CO_2 from apatite lattice due to carbonate decomposition <800: features such as osteons remain visible; carbon formed from organic material bonds with oxygen to form CO_2; calcination occurs 800: shrinkage in granular form of the spongy bone	700: formation of larger, rounded crystalline structures <700: sudden growth of HA crystals; mineral phase replaced by large clumps of crystallites <800: loss of homogeneity in bone's crystallinity 600-900: average crystallite size 256-1500Å 800: crystal size reaches maximum	800: beginning of fusion of individual crystals to form larger ones	>800: birefringence ceased 700: $\delta^{13}C$ (collagen) = -22‰ $\delta^{15}C$ (collagen) = -1‰ $\delta^{13}C$ (carbonate) = -11‰ $\delta^{15}C$ (carbonate) = -17‰ 800: $\delta^{13}C$ (collagen) = -21‰ $\delta^{15}C$ (collagen) = -2‰ $\delta^{13}C$ (carbonate) = -10‰ $\delta^{15}C$ (carbonate) = -17‰ 900: $\delta^{13}C$ (collagen) = -23‰ $\delta^{15}C$ (collagen) = -3‰ $\delta^{13}C$ (carbonate) = 17‰ $\delta^{15}C$ (carbonate) = -17‰	700: able to still retrieve amplifiable mtDNA from cremated samples 800: no mtDNA able to be amplified/replicated	700: 0.3 wt.% 800: 0.2 wt.% 900: 0.2 wt.%	700: violet	600-900 'Inversion'
<940: particles of previous stage melt and coalesce into larger structures 1000-1200: 14-18% shrinkage observed	White (calcined) Light blue-grey Light grey	>800: osteons appear to infold, and blisters or large oval openings are observed >900: complete loss of osseous structures and a loss in prominence of vascular canals 1100: lacunae gradually disappear; cracks that had crossed between osteons turn into large fissures >1600: complete destruction of all bone's structural features due to melting and recrystallisation of the bone mineral upon cooling; osteons and Haversian canals remain intact until recrystallisation occurs where they then change their shape and size with increasing heat exposure	>800: formation of new hexagonal crystals with a prismatic habit, increasing in size with temp rise; fusion of crystals due to sintering >900: granulose structure with amorphous crystal clusters >900: average crystallite size >1500Å	>800: sintering or fusion of bone salts and HA crystals occurs 900: crystals melt to significantly smaller, rounded granular structures >1000: localised fusion of the hexagonal crystals resulting in crystals of varying morphology and sizes; granular structure has completely disappeared giving rise to a compact surface of microcrystals	1000: $\delta^{13}C$ (collagen) = -23‰ $\delta^{15}C$ (collagen) = -1‰ $\delta^{13}C$ (carbonate) = -8‰ $\delta^{15}C$ (carbonate) = -16‰ 1000: stable isotope values of strontium (Sr) remained unaltered up to 1000°C	-	>900: 0.1 wt.%	-	>900 'Fusion'

Adapted from (17, 43, 44, 46, 49-53, 58-65)

4.2.2 Duration

The duration of a burning event has a direct effect on several factors determining the final appearance of heat-altered bone. Primarily, the loss of moisture and collagen increases with prolonged heat exposure. This is evident in commercial cremations, as longer durations are required for larger individuals, due to increased amounts of body fat and muscle (41). The duration of a burning event will also affect the colour changes exhibited on bone, though this factor alone has less influence on colour change than other variables, such as temperature and body composition (66). The shorter the burning event, the less likely the bones are to exhibit significant colour changes, such as the white of calcination. However, shorter durations can produce calcinated bones if the temperature is very high. Some studies have demonstrated that bones burned at extreme temperatures for shorter durations show similar effects to those burned at milder temperatures for longer durations (66). These variations in fire intensities and durations also pose a challenge to forensic investigators in terms of identifying remains, which becomes increasingly difficult with longer durations (67).

4.2.3 Composition of remains

Fleshed remains

When a fresh body is burnt, soft tissues act as insulators to the underlying bones, protecting them from the heat. This effect is known as tissue shielding, as the skin, connective tissues, and muscles must first burn away before contact with the bones can occur (41). If the fire is short-lived, the bones may only show minimal heat-related effects or remain completely unaltered. There are several factors that affect the rate of destruction of underlying bones when soft tissues are still intact: the size of the body or the anatomical region (e.g., a foot in comparison to a thigh), the tissue thickness and distribution, the location and the position of the body in relation to the fire, and whether or not

the body assumed the “pugilistic posture” during burning. Greater degrees of heat-alteration will occur to bones that are covered by only small amounts of tissue, such as the frontal bone of the cranium, in comparison to bones that are covered more substantially, such as the femur. A larger body, with a higher mass of fat tissue, will also delay the heat-related effects to the bones, as there is more tissue to burn through before they can be accessed (41, 45). An overview of the progression of heat alteration when remains assume the pugilistic posture is provided in Figure 1.

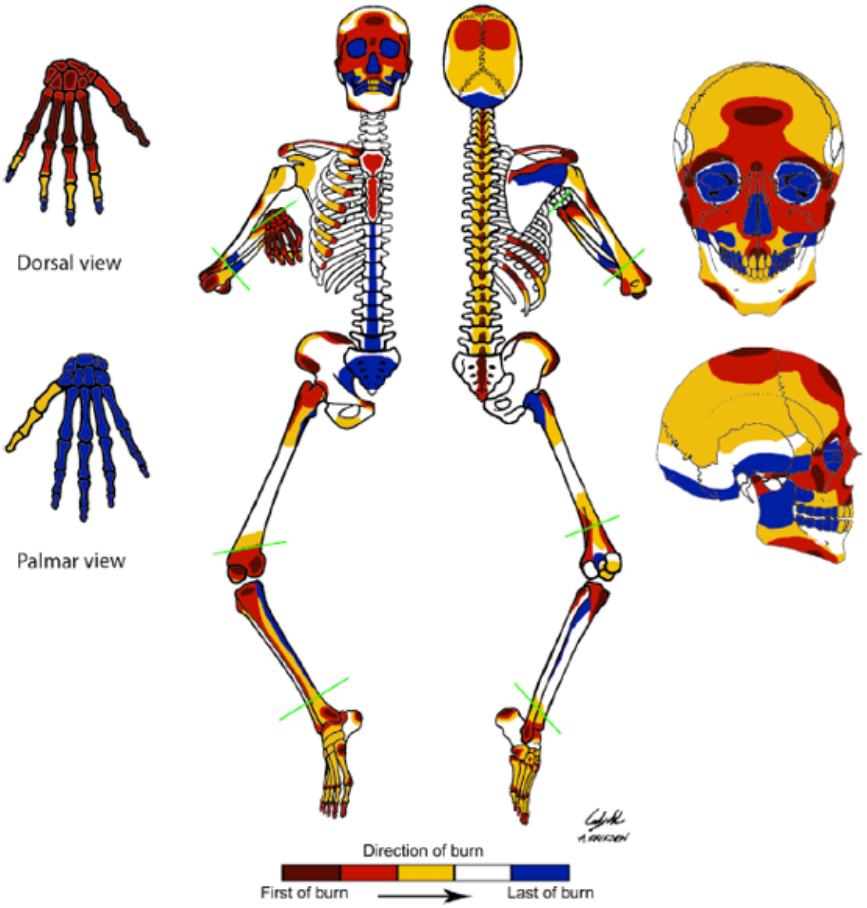

Figure 4.1. Anterior and posterior views of the human skeleton assuming the pugilistic posture. The primary, secondary and final areas to burn are highlighted according to the colour scale provided. Dorsal and palmar views of the hand are shown, along with magnified views of the typical burn patterns on the skull. The green lines indicate areas where burn fractures are most likely to occur. (45)

The development of the pugilistic posture will occur when fresh remains are subjected to intense heat. As the fire rapidly dehydrates and shrinks the flexor muscles, they begin to contract, resulting

in the extreme flexion of the postcranial elements, most noticeably in the upper and lower appendages (41, 45, 46). This flexion leads to the increased exposure of some elements, whilst others become more protected through tissue shielding. Areas like the wrists, elbows and knees are first affected by this phenomenon, resulting in differential burning, with the upper, more exposed portions displaying increased damage (41, 45).

Further evidence of tissue shielding, and the burning of fleshed remains is seen in the presence of a heat border and cracking at the heat line. A heat border is an off-white area of bone where the soft tissues have started to recede, and where the heat-alteration of bone begins. Adjacent to this border is the heat line, which marks the transition from burned to unburned bone (41, 43, 45). It is not uncommon to see the entire spectrum of heat alterations on a single bone, from fresh bone to charred and calcined portions due to the variations in tissue depth and body posture during burning (Figure 4.2). These variations can aid investigators in differentiating between the burning of fleshed or dry bone (46). Fleshed bones will also display severe shrinkage, warping and cracking due to the intense heat rapidly removing all moisture and collagen, in addition to the forces applied when the muscles, ligaments and tendons shrink away from their attachment points (41, 46).

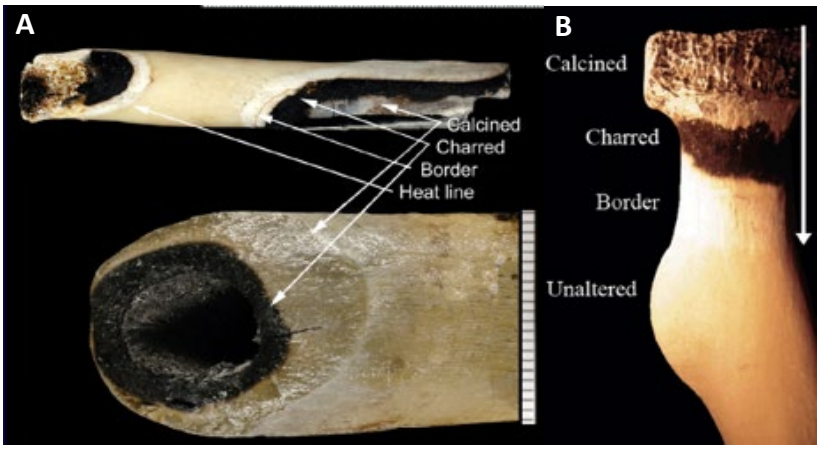

Figure 4.2. A: Differential burning patterns shown in a radius shaft (top) and mid-humerus shaft (bottom). B: Patterned colour changes on a proximal radius showing the direction of heat alteration (white arrow). From (67).

Decomposing remains

The stage of body decomposition during burning will affect the appearance of the bones when recovered. A very slightly decomposed body will react much the same as a fresh body, exhibiting the same fracture patterns and burn morphologies. In contrast, a more heavily decomposed body will display abnormal burn patterns. This is due to the breakdown of muscles, skin, tendons, and ligaments, meaning that the body may not have assumed the typical pugilistic posture. This will result in scorched bone fragments that may otherwise have been protected during formation of the pugilistic posture. The abnormality of the resulting burn pattern can aid forensic investigators in estimating the PMI of the remains (41).

Fresh or dry bones (unfleshed)

Bones that are not encased in soft tissues follow a more homogenous burn pattern. They will often reach calcination faster than fleshed bones, with minimal cracking and warping as most of the moisture content had already evaporated prior to the burning event. Longitudinal fractures are still common as the bone dries further and the collagen content is gradually removed, though many of the other effects seen in fleshed bones are not apparent (41, 46).

4.2.4 Teeth

Not as much research has been conducted into the effects that varying temperatures have on the taphonomy of teeth in comparison to bone. In the forensic context, teeth can be used to identify an individual when other means of identification (e.g., visual) are not viable. This is often the case when remains are burnt beyond recognition, or when only bones and teeth remain (68). The teeth enamel

and dentin have a lower organic component, and a different microstructure than bone (e.g., rods instead of osteons), which contributes to their post-mortem survivability in the environment (69). Despite this difference, teeth progress through the same general stages of heat-alteration, including carbonisation and calcination (41). Dentin will often exhibit these changes faster than enamel, due to their differing levels of mineral content, meaning one may show signs of carbonisation when the other appears unaltered. This can also be seen with fires that burn out quickly, as there will be charred soft tissues adhering to the teeth, though the enamel may be completely unaltered (68). The fracture patterns identified in heat-altered bone are also evident in burnt teeth specimens. Roots will often display transverse and step fractures, while enamel may display longitudinal cracks along lingual, central and labial grooves (68).

The colour changes observed at varying temperatures in both enamel and dentin are also similar to those seen in bone and are summarised, along with the general histological and morphological changes, in table 4.2.

Table 4.2. Physical, chemical, and biological changes to teeth associated with increasing thermal alteration

	Physical Characterisation				Biological characterisation	Temperature (°C)
	Macroscopic appearance	*Colour*	*Microstructure (histology)*	*Crystallinity*	*DNA*	
Enamel	100: longitudinal fissures observed 185-285: longitudinal and transverse clefts of enamel; onset of enamel fracture; separation between enamel and dentin 204: slight enamel flaking seen around CEJ 260: crown enamel beginning to separate from root structure	White (natural) Yellowish-white Yellowish brown Pale brown Translucent at CEJ	185-285: development of surface dimpling, but overall surface texture is smooth	No change in crystallinity	Viable DNA extracted, though mean DNA concentrations decreased as temperatures increased 100: similar DNA concentrations to unburnt teeth >200: DNA concentrations significantly decreased	<300 'Dehydration'
Dentin	<185: dentinal surface of the pulp cavity normal and unaltered	White (natural) Yellowish-white Dark reddish-brown	185-285: peritubular matrix visibly shrunken and separated from the intertubular matrix; surface of intertubular matrix shows many small asperites that give it a roughened appearance	<185: calcospherites typical of the growing dentinal surface are clearly visible and are pierced by a regular array of smooth-edged, circular openings to the dental tubules		
Root	100-200: longitudinal and transverse cracks	Yellow				
Enamel	371: glossy appearance making it appear metallic 371-472: many small, non-uniform cracks over majority of enamel (patina-like appearance) 400: longitudinal and transverse fissures; more evident separation between enamel and dentin 300-538: crown integrity severely compromised and may entirely separate from roots; enamel disintegrated into many small fragments 482: glossiness gone >500: calcination observed	Dark greyish-brown Very dark brown Black (carbonisation) Light grey (calcination)	285-440: rounded particles appear, covering surface >440: surface marked by appearance of vitrified or glassy particles separated by many pores and fissures	No change in crystallinity	400: Viable DNA found to withstand this temperature for up to 1hr, though of much lesser quality and quantity than lower temps; <0.05ug/mL extracted >500: Very difficult to extract viable DNA; <0.05ug/mL extracted	300-600 'Decomposition'
Dentin	400: longitudinal and transverse fissures; more evident separation between enamel and dentin	Black	285-440: asperites have melted and smoothed out; division between peritubular and intertubular matrix is only infrequently visible; intertubular matrix forms a network of bars between opening of dental tubules	285-440: opening of dental tubules are elongated, rather than circular		
Root	400: root fragmentation observed in some teeth	Olive-brown Light grey/grey				
Enamel	No further significant morphological changes observed*	Remains light grey; No further colour change*	No significant histological changes observed for this stage	No change in crystallinity	>600: <0.05ug/mL extracted	600-900 'Inversion'
Dentin	No further significant morphological changes observed*	No further colour changes observed*	440-800: appearance of many particles, giving the surface a frothy or fleecy texture; some areas of glassy texture, perforated by irregularly shaped openings	440-800: elongation and enlargement of the tubule openings		
Root	700: root separation observed	No further colour changes observed*				
Enamel	No further significant morphological changes observed*	Remains light grey; No further colour change*	800-940: fine particles of previous stage coalesce into larger, smoother-surfaced globules that fuse into an irregularly shaped mass pierced by rounded holes	800: enamel rods present an altered structure		>900 'Fusion'
Dentin	No further significant morphological changes observed*	No further colour changes observed*	800-940: frothy protuberances have coalesced into globules that fuse into nodular spikes arranged in a staggered array; spikes are remnants of the intertubular bars	800-940: spaces between spikes are remnant of tubules		
Root	No further significant morphological changes observed*	No further colour changes observed*				

No information available; further changes could be apparent, though more research in this area is required; Adapted from (44, 67, 69-71)

5. TAPHONOMY IN AQUATIC ENVIRONMENTS

Aquatic environments include both freshwater (fluvial, lacustrine, groundwater etc.) and saltwater (marine) systems, where remains can be deposited as a result of homicides, suicides, accidents or burials at sea (72, 73). Whilst aquatic environments may become a secondary depositional location for remains, only those deposited into aquatic environment as a primary location will be considered in this review, due to a lack of research surrounding the taphonomic histories of remains deposited secondarily. Artificial environments, such as suitcases, bags, and situations where bodies have been weighted down or mutilated in any way, will be excluded from this review due to the complexity of the described scenarios, and what little current knowledge is known surrounding their decomposition rates and taphonomic histories.

5.1 Decomposition in aquatic environments

The decomposition of human remains progresses slower in aquatic environments in comparison to surface or burial environments. Remains that are fully submerged restrict access to terrestrial decomposing organisms, such as flies, maggots, and large scavengers. Some terrestrial invertebrates, such as flies, will be able to colonise the portion of a body that is above the water level, whilst aquatic invertebrates colonise the submerged portion. If consumption of the exposed soft tissues does not occur, the remains may become mummified, whilst the submerged soft tissues continue to decompose (72). Other factors affecting the rate of decomposition in aquatic environments include the size and the position of the body, water salinity, any present trauma, decreased water temperatures - which inhibit bacterial action -, and water flow and movement rates (72, 74). Remains may

be carried large distances before complete decomposition and disarticulation occurs, even losing body parts along the way (75, 76).

Bodies deposited into aquatic environments will undergo a consistent sequence of decomposition before skeletonization and disarticulation. This sequence includes submerged fresh, early floating, floating decay, advanced floating decay, and sunken remains (74). Other processes that are seen during aquatic decomposition include the development of 'washer women's skin' and skin discolouration through marbling, skin slippage, and a complete loss of hair, skin and nails (72). The complete loss of soft tissues generally takes around one month, unless protective items, such as heavy or tight clothing, or shoes, prevent tissue loss (75). Once disarticulation commences, it will proceed from the most distal joint to proximal, with elements like the mandible, cranium and hands separating first. The arms, legs and feet are disarticulated next, followed by the pelvis and thighs, which tend to subsist longer before removal (77). The formation of adipocere is also common in aquatic environments where the temperature is cooler and anaerobic bacteria abundant, especially in anoxic waters (74).

5.2 Freshwater (rivers, lakes, wetlands)

5.2.1 Transport mechanisms

Fluvial systems can transport remains across great distances, even depositing them into the ocean (76). Transport is generally episodic due to water level fluctuations, and the remains can potentially become embedded in the riverbed or trapped with other structures, such as logs, rocks, or debris. Like bodies, bones also go through a cycle of flotation and sinking, depending on how and when the remains were deposited into the

water. Complete bodies have a similar density to that of water; therefore, they will initially float on the surface and will be more easily transported downstream. As the body progresses through each stage of decomposition, the remains will periodically float and sink until they reach the advanced decomposition stage. If bones are deposited directly into the fluvial environment, they will float or sink depending on the currents and water movements. These varying rates and mechanisms of transport result in different taphonomic alterations of bones, as they may be affected differently by other taphonomic agents such as bioerosion and sediment abrasion (72).

5.2.2 Sediment abrasion and embedding

Depending on the mechanisms and force of transport, different levels of sediment abrasion may be seen on bones. Rounding and smoothing of any protruding edge is commonly observed, along with small scratches, pitting, chipping, and denting of the cortical surface. Long grooves and fractures are rarely seen, though can be produced if the force of movement of the element is strong enough. Natural openings within the bone, such as vascular canals and foramina will gradually become enlarged over time, and in juvenile remains, epiphyses may detach if not fused. As the cortical layer thins, the underlying trabecular bone may also be exposed towards the epiphyseal ends. The rate and degree of damage is also influenced by the type of sediment and environment within the fluvial system. Rivers with large, coarse sediments will impart increased levels of damage upon bone, whilst those with fine sandy or silty sediments will produce more subtle damage. These sediments can also become embedded within bone, into any natural openings, grooves, pits, or exposed trabecular bones from the processes of

sediment abrasion. This sediment impaction can provide information to investigators as to where the remains were deposited at one point in their taphonomic history (72).

5.2.3 Bioerosion

In the fluvial environment some species of plants and animals will selectively adhere to bone, leaving traces that can be identified at recovery. Algae are amongst the first to colonise bone, primarily those in shallow waters where sunlight is abundant, the euphotic zone. Bryozoa are also known to colonise bone, both in fluvial and marine settings. Bryozoans are colonial filter feeders, that will adhere to bone in colonies of individual zooids, protected by a 'shell' of organic or mineralized components, such as calcium carbonate. The growth rate of these organisms may assist in the determination of the minimum immersion time of the bone (76). Traces of larval boring can sometimes be observed on bone recovered from fluvial settings and will present as smooth-walled troughs with a U-shaped cross-section. Invertebrate consumption of bone will leave similar traces due to feeding on the cortical layer and can often be mistaken for the pitted appearance of acid etching. However, these feeding traces are deeper and more regular than the haphazard pitting left by acid etching (72).

5.2.4 Discolouration

The discolouration of bones in a fluvial environment can be caused by a variety of factors. The most common colour change is from the bone's original neutral white colour to an off-white or light brown. This is due to the complete or partial burial of the

bone in the fluvial sediment. The degree of brown discolouration will depend on the composition of the sediment, which can range anywhere from pebbles to a sandy silt or mud. Other common colour changes are the result of interaction with fluvial water plants or of the natural processes of decomposition. A light green staining has been observed accompanying the growth of algae on the bone surface, and black staining has been seen with adipocere formation. A yellow staining has also been noted as fat leaches from the bone's interior due to decomposition (72).

5.2.5 Water pH

Not much research has been conducted regarding the effects of varying pH levels of water on bone structure, and whether or not pH would play a significant role in the degradation of bone in a fluvial environment. Acid etching upon bone is a known phenomenon, occurring on bones deposited in acid-rich soils. Acid etching in a fluvial setting appears as pitting and roughening on the surface, often accompanied by discolouration. The rate of acid etching in a fluvial environment is not well studied, though the constant movement of remains in water, in comparison to terrestrial remains that are stationary and in direct contact with acid-rich soils, would suggest that acid etching occurs at a much slower rate in fluvial settings (72). An experimental study conducted using pre-cut sections of long bones placed into containers of varying pH solutions assessed the impacts that pH had over a year-long period. The best levels of preservation were seen at pH 7 and 10, whilst the poorest preservation was seen at pH 1 and 14, with the specimens completely dissolved after just 3 weeks at pH 1. Adipocere was formed within the marrow cavities of solutions at pH 4, 7 and 10. This study

demonstrates that fluvial systems of extreme pH levels may degrade remains faster than those of more neutral pH levels, meaning different considerations would have to be made regarding the taphonomic histories of these specimens, and subsequent PMSI and PMI ranges (78).

5.3 Saltwater

5.3.1 UV exposure

Bones deposited into the marine environment will experience increased levels of bleaching over time, due to a chemical reaction between the UV light from the sun, and the salt water, alongside the gradual loss of organic content (73, 76). Marine bleaching affects the entire surface of a bone simultaneously, rather than just the upper exposed portion, as is seen in subaerial environments. This is due to the bone constantly moving in the marine environment, providing access to its entire surface (73, 74). Bleaching may not be initially obvious on bones recovered from marine environments, due to being obscured by adhering taxa, sediment, or staining. Staining can occur in the form of reddish or dark brown/black discolourations from iron oxides and iron sulphides, respectively (74, 76). Staining can also be the result of the oxidation of metallic alloys in contact with the bone, such as those containing copper, iron, manganese or zinc (75, 79). Any underlying bleaching can be uncovered through light abrasion of the bone, either whilst still in the marine environment, or after recovery of the element (73). Marine bleaching can be differentiated from subaerial bleaching through analysis of other taphonomic effects present on the bone. Subaerial bleaching will often be accompanied by cracking, flaking, and exfoliation of the cortical surface, with only a

portion affected by UV exposure. In comparison, marine bleaching will be uniform, with no accompanying cortical damage (73).

5.3.2 Bioerosion

Various marine taxa will adhere to hard substrates, such as bone, for support or to feed upon other adhering taxa or on the bone itself (73, 74, 76). The presence of adhering marine taxa on bone reinforces the assumption that the remains were within a marine depositional environment at one stage of their taphonomic history. This is especially apparent when the remains were recovered from another depositional environment, such as a burial or subaerial location. Some marine taxa can also be used in the estimation of PMSI and PMI ranges, based on their degree of growth (73). Often, the marine taxa may be long gone when the remains are discovered, though evidence of their attachment or feeding habits can still be observed. Bioerosion is the term used to describe the degradation or removal of bone by living organisms that have used the bone as shelter, support or as a nutrient source. This term also extends to any damage caused to bone by living organisms feeding on the adhering soft tissues, or other organisms present (73, 74).

Common adhering marine taxa known to utilise the hard substrate of bone include algae, coral, Bryozoa, molluscs and barnacles. Algae and Bryozoa will colonise hard substrates in both freshwater and marine environments, providing evidence that the remains were deposited in an aquatic environment. These species are easily identifiable, especially bryozoans, which may leave behind a hard organic or mineral ‘shell’ on bone once the organism has died. Coral, molluscs, and barnacles are all

marine-dwelling organisms, who will often adhere to the hard substrate of bone as larval stages to allow the adult form to grow. Their presence indicates that the remains were deposited into a marine environment, specifically, and can be identified through their hard calcium carbonate shells, feeding and homing scars, and calcified plates. Whilst these organisms often do not produce damage to bone, their presence, or traces, indicate that the remains were deposited into an aquatic environment at one point in time. This is especially useful if the remains were found outside of the aquatic environment, and can assist the investigators to infer a taphonomic timeline (73, 76).

Another form of bioerosion commonly seen on bones deposited into a marine environment includes microboring and invertebrate grazing traces. Microboring is described as the formation of small tunnels on and through bone due to the feeding action of some marine taxa (80). Common microborers include *Osedax* worms, osteophagous crabs such as the tanner crabs, fungi, bacteria, and algae (73, 74, 76). Many of these species leave behind characteristic traces. *Osedax* and *Polychaete* worms utilise heterotrophic bacterial endosymbionts or proteolytic enzymes, respectively, to break down the complex organic components of bone for feeding (81). This activity leads to the formation of tunnels through bone, though these worms will also produce tunnels as a form of protective housing (76). These worms typically inhabit deep water below 100m depth and often occur in high densities (73). Fungi, bacteria and algae commonly produce Wedl-type tunnelling, and also act as a food source to larger species, which encourages grazing upon bone (73). Bones may also show the presence of foraminifera within pore and trabecular spaces (Figure 5.1), which are single-celled organisms that are ubiquitous to marine environments (79).

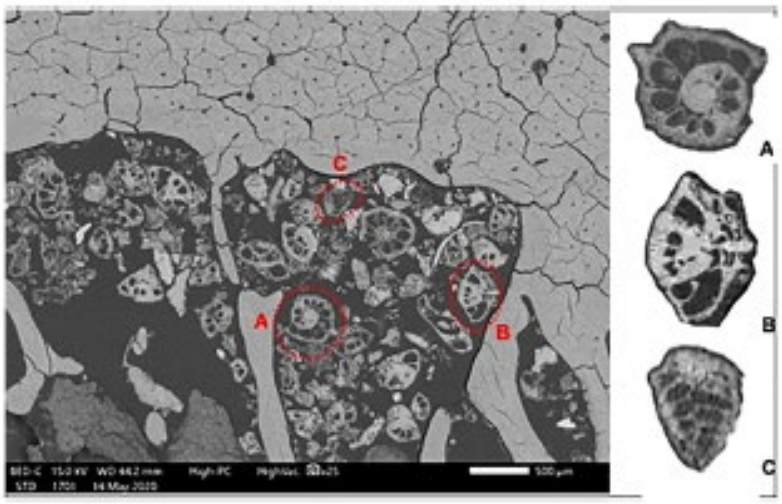

Figure 5.1. Foraminifera species found within a bone sample from the Zeewijk shipwreck of 1727. Most of the foraminifera in this image are *Neotalia calcar* (A-B), with a minor presence of species from the genera *Reussella* or *Fijella* (C). From (79).

When marine organisms, predominantly invertebrates, feed on bone, they leave behind characteristic traces of their activity, which can often indicate a particular species. Osteophagous crabs, such as the tanner crab, have been observed on bone in deep-water environments, often feeding on *Osedax* worms inhabiting the bones. These crabs may produce superficial grooves or regular indentations, though most commonly a general erosion of the thinner areas of bone occurs (73, 81). Molluscs not only adhere to bones but will also feed on them as a secondary nutrient source. They use a chitinous feeding appendage lined with small teeth called a radula, which scrapes away at the bone surface leaving behind a series of parallel striations approximately 20-100µm in depth. These striations occur in blocks and can cover large surface areas. Sea urchins, or echinoids, use their five-toothed jaw apparatus to remove bone, resulting in a star-shaped pattern of grooves along the bone surface (73).

Vertebrate marine species are also known to damage bone, predominantly through consumption of adhering soft tissues. Sharks will leave punctures, fractures, furrows, and striations. Marks may be overlapping or parallel, depending on how many times the multiple rows of teeth have passed over the bone. It is unclear however whether these marks are the result of hunting or scavenging activities (73).

5.3.3 Sediment abrasion and embedding

The constant and highly energetic movement of coastal waters can lead to the impaction of bones against multiple substrates, resulting in characteristic damage (73, 76). As bones come into contact with the ocean floor sediment, or sharp, rocky areas, their surface will gradually accumulate damage, such as rounding, cortical thinning which may expose underlying trabecular bone, loss of unfused elements like epiphyses, and surface damage like scratches, pitting or indentations (Figure 5.2)(74, 76). Any natural openings within a bone, like vascular canals and foramina, will gradually expand in a process called “windowing” (76). The rate of abrasion will generally depend on the composition of the sediment surrounding the bone. Coarser sediments have been demonstrated to produce greater abrasive damage over shorter time periods than finer sediments, though the degree of damage caused by these sediments is also dependent on the duration of exposure, abrasive force, bone hardness and elasticity as well as the sphericity of the sediment grains (74). Sediment abrasion can also expose prior taphonomic effects on bone, such as subaerial weathering characteristics like cracking and bleaching, which may have been obscured by other elements in the marine environment (76). Sometimes bones can be so abraded that they can be easily mistaken

for common ocean detritus like drift-wood or coral, making recovery more difficult (74). Whilst the effects of abrasion on bones in marine environments is generally known, an exact timeframe for these processes has not yet been established (73, 74). A general summation of the stages of abrasion determined experimentally are provided in table 5.1.

Bones deposited into a marine environment can also undergo sediment embedding similar to those seen in freshwater environments, though the sediments available for embedding will differ (73, 76).

Figure 5.2. Anterior view of distal radii of white-tailed deer showing marine sediment abrasion from stage 0 (left) to stage 4 (right) after variable durations of tumbling with sand. Stage 0 shows no rounding of margins. Stage 1 shows rounding of margins. Stage 2 has the beginning of trabecular bone exposure. Stage 3 has more advanced trabecular bone exposure and more extensive rounding of margins. Stage 4 shows the most severe trabecular bone exposure and loss of margins (reshaping of bone outline). Note: Scale is in cm, and all results are from experimental research. From (74).

Table 5.1. Experimentally determined abrasion stages for bones found in a marine environment

Stage	Description
0	Natural; soft tissue may still adhere
1	Slight rounding of margin seen; soft tissue may still adhere
2	Moderate rounding of margins seen; minor trabecular bone exposure appearing on outermost projections of bone; dense soft tissue may still adhere (e.g., ligaments, tendons, cartilage)
3	Heaving rounding of margins; significant trabecular bone exposure on both outermost projections and broader margins; basic bone outline and contours of surface still visible; adhering soft tissue generally no longer present; perimeter outline of bone still present though modified
4	Severe rounding on bone margins with extremities removed entirely; may show exposure of marrow cavity and/or loss of most surface features; perimeter outline of bone more significantly altered

Adapted from (74)
Note: fracturing to bones may occur in any stage, even to stage 0 bones

5.3.4 Isotopic and REE concentrations

The isotopic composition of bones can be used to reconstruct factors such as perimortem diet and migration behaviours, as well as the post-mortem depositional environment (82, 83). Certain isotopes obtained from BA within bones can provide information regarding the climatic and environmental conditions of temperature and humidity that affected the bones during deposition (82, 83). These isotopes are incorporated into the BA through recrystallisation, as the bone undergoes natural diagenesis (83). This diagenetic alteration takes many years to occur to a point where levels are altered enough to show a clear difference between the natural bone isotopic concentrations, and concentrations reflecting the surrounding depositional environment (83). However, when this process occurs under microbially-mediated conditions, this rate is increased, resulting in the diagenetic alteration process taking a few hundreds of years rather than a few thousand (83).

Rare earth elements (REEs) can similarly be used in the identification of a bone's taphonomic history, as REEs concentrations are reflective of a particular environment and geographical location, creating a unique diagenetic 'signal' (38). Bones that are deposited in a specific environment for long periods of time will eventually reflect an identical REE ratio to that of the surrounding pore water (83). This uptake of REEs from the surrounding environment begins early in the diagenetic process, but can take hundreds of years to match the ratios of the depositional environment (72, 73). Typical REEs are Hf, U and Th, whereas more common elements of Ca, Mg, Fe and Zn are predominantly controlled by biogenetic processes within the living body (38). REEs are often incorporated into BA when the decay of the organic component (collagen) leads to an increase of bone porosity. Once the BA crystal structure becomes exposed, it is susceptible to recrystallisation and increased uptake of REEs from the surrounding environment (83). Variations in chemical abundances are less pronounced in aquatic environments, and differ even between fluvial and marine settings, which should be taken into account when assessing REE ratios as a form of taphonomic analysis (73, 84).

5.3.5 Salt crystal formation and drying

When bones wash up onto land after long periods of aquatic submersion, they will begin to progress through the typical stages of subaerial weathering. However, there are several differentiating factors between bones that have only undergone subaerial weathering and those that were first submerged for a relevant amount of time before being deposited on land. Bones submerged in a marine environment for long periods of time will accumulate a large amount of salt ions into their porous matrix. Once the bone

begins to dry out, salt ions begin to recrystallise, resulting in the expansion of the bone matrix and formation of large cracks. These cracks can mimic those created in a subaerial weathering environment, though the large concentration of salts on and within the bone will aid in differentiating these two fracture patterns (73). Fractures can also be formed along the bone's surface from the rapid evaporation of moisture. This drying process causes the bone matrix to contract, creating extensional stresses along the surfaces. This can lead to the formation of deep, elongate cracking that extends from the cortical surface through to the medullary cavity, and often along the entire length of the diaphysis in the case of long bones. Depending on the speed of water evaporation from bone, warping can occur. Bones are somewhat flexible when completely hydrated, meaning that their physical shape can be altered. If the shape of a bone is altered from its normal anatomical position when drying, it will retain this shape (72). The overall appearance of a submerged bone's surface is different to that of a subaerially weathered bone, presenting a dull and chalky texture. This is likely due to the evaporation of moisture and crystallisation of absorbed marine salts, leaving behind small salt and mineral particles on the bone surface (76).

6. TAPHONOMY OF FROZEN TISSUE

Frozen environments define those with an ambient temperature below 0°C (32°F), often with the presence of ice or snow, for part or all the calendar year. Human remains found in these environments are often the result of accidental deaths, such as when hiking or because of a plane crash, due to the remoteness and harsh nature of frozen landscapes (85).

6.1 Decomposition

Temperature is one of several extrinsic variables that has a direct influence on the rate of decomposition. Frozen environments typically consist of temperatures below freezing (0°C), which significantly slows the decomposition process (85). Bacteria thrive in a warm, moist environment, with an ideal temperature close to that of the human body (37°C). In a frozen environment, their activities are greatly reduced, leading to excellent soft tissue preservation, and in some cases mummification of remains (85-87). In comparison to remains in temperate environments, that decompose from the inside out also due to endogenous microorganisms, frozen remains tend to decompose, if at all, from the outside in. This is due to the loss of internal microorganisms, reduced anaerobic activity, and accelerated external decay through the function of necrophagous insects. The freezing of tissues and cells causes the skin to take on a dull grey colour, and reduces its overall mechanical integrity, making access easier for insects and external microorganisms (86, 88). Some studies on ancient woolly mammoths recovered from permafrost regions have shown excellent soft tissue preservation, with ligaments, cartilage, fat deposits, and entire brains recovered (87, 89).

6.2 Glacial environments

Glacial environments are very dynamic, consisting of multiple moving layers of ice and rocks. Human remains embedded within glaciers are subject to damage caused by glacial movement, load, and any surrounding materials. These elements can abrade, polish, pulverise, and chip at skeletal material, and can expose underlying trabecular bone as the outer cortical bone is damaged, removed and lost. The location of remains within a glacier

also affects their taphonomic history. Remains that are exposed for days or months throughout the year, due to freeze-thaw cycles, are more likely to show evidence of weathering, scavenging activity, and accelerated decomposition, compared to those that remain completely buried within ice, where excellent soft tissue preservation is observed. Remains can also be dispersed a great distance from where they were initially deposited, due to glacial movement (85).

6.3 Freeze-thaw cycles

When water freezes, it can increase up to 9% of the original volume, as ice crystals are formed. The formation of ice within the small bone spaces not only removes moisture from the bone matrix but can also lead to structural weakening and the formation of microcracks. When this ice melts, and refreezes with subsequent freeze-thaw cycles (e.g., due to seasonal fluctuations), it leaves more space to be filled within the bone, leading to a further expansion and structural damage (90). In Tersigni (2007) (91), microfractures were identified within the Haversian canal systems of frozen bone samples, absent from all controls. Turpin (2017) (86) also noted these microfractures in samples of human bone frozen for 21 days. Two distinct types of microfracturing were observed, including irregularly shaped transverse cracks along the interior cortical edge, and linear osteonal cracks originating in the Haversian canal system of a single osteon. Of interest was the lack of microfracturing seen in human bones that had been embalmed prior to the freeze-thaw experiment (86). Whilst research surrounding the taphonomy of teeth in frozen environments is scant, one study (87) found mammoth teeth to be intact and without any post-mortem damage after thousands of years buried beneath the permafrost.

6.4 Freeze-drying

When remains undergo rapid freezing and drying, they are essentially preserved in mummification, because the process of decomposition ceases (86). Rapid freezing removes moisture from the intracellular and extracellular spaces within the body, as it is transformed into ice crystals. This results in the dehydration of tissues and subsequent mummification of the remains (88). With little interference from the standard taphonomic agents found in many other depositional environments, such as increased temperatures, weathering, and insect or animal activities, remains can essentially persist in a mummified state until recovery (88).

7. DISCUSSION

Forensic taphonomy, a sub-discipline of forensic anthropology, studies the post-mortem modifications which affect human and non-human remains, and are produced by a variety of taphonomic agents of physical, chemical and biological nature (10). In this study, the current literature was reviewed to compile to-date information on the taphonomic agents present across five depositional environments, and the effects generated on biomineralized tissues (bone and teeth). The aim was to assist the forensic community in reconstructing the post-mortem histories of remains in forensic investigations.

Whilst some environments include similar taphonomic agents, other taphonomic effects are unique to specific depositional environments. Burial environments, in which deceased individuals are deposited into a grave, either a formal coffin burial or the placement into a clandestine pit, featured a few key taphonomic effects (14). Buried biomineralized tissues may exhibit evidence of bioerosion (e.g., by bacteria), degradation due to soil characteristics

(e.g., pH), mineral staining from soil particulates or coffin hardware, and coffin wear. Subaerial environments, with remains placed at the ground surface and exposed to the natural elements (30, 31), are associated with characteristic effects, such as weathering from fluctuating climatic conditions, delamination, cracking, bleaching of exposed surfaces due to increased UV exposure, and bioerosion (e.g., by plants). Burnt remains will present different taphonomic effects (41) depending on the range of temperatures and the duration of the fire, as well as the composition of the body (i.e., decomposed, fresh, skeletonised). Bone and teeth may exhibit intense colour changes from an initial pale yellow, through to brown and black (carbonised) and white/grey-blue (calcined). Heat-related fractures often accompany colour changes and are linked to a rapid reduction of the tissues' organic component. Histological and crystallographic analyses may also show changes in the micro- and nanostructure of bone and teeth. Frozen environments are defined by an ambient temperature below 0°C (32°F), often with the presence of ice or snow, for most of the calendar year (85). Taphonomic research in this area is limited, however common factors affecting bones and teeth include freeze-thaw cycles, mummification due to the decreased temperature reducing or stopping decomposition, and damages linked to patterns of movement and soil composition typical of glaciers. In aquatic environments, biomineralized tissues may undergo several characteristic alterations depending on the type of water they are deposited in, that is freshwater or saltwater. Common effects observed in freshwater include sediment abrasion, degradation by water lower pH levels, and discolouration. Similarly, in marine environments, bones and teeth may undergo complete bleaching (as opposed to the partial bleaching seen in subaerial environments), bioerosion (e.g., from adhering taxa), altered isotope and REE concentrations, and sediment abrasion (72, 73).

When biomineralized tissues are recovered from one of the listed environments, the first step is to determine the human or non-human species. This is usually achieved by forensic pathologists and anthropologists, who are trained in recognising human bones and teeth. Non-human bones can be commonly mistaken for human bones (e.g., a bear's paw) (8). This is especially evident when remains have been scattered by scavenging activities. Once the human species is confirmed, the next steps aim towards personal identification and the assessment of the circumstances surrounding death, including the estimation of the PMI. Forensic investigators employed by the local law enforcement will conduct the investigation surrounding the circumstances of death, how was the recovery location reached, and the PMI. The forensic anthropologist will produce a biological profile, with the determination of sex, age, living stature and pathological traits, which may lead to either a presumptive or a positive identification. Whilst forensic taphonomy can similarly provide an insight into the circumstances surrounding death and an estimation of PMI, it can also supply information about the post-mortem history of the remains. By understanding the environment-specific agents that damage bones and teeth, a more detailed analysis of the depositional environment can be produced. The location and/or the timeline of the deposition of the remains may not match other evidence collected in the investigation, therefore a thorough analysis of the taphonomic damage to bones and teeth may detect and explain the differences (92). This information may then assist investigators by narrowing down the location and the time of deposition. Forensic taphonomy can also provide insight into the analytical techniques that can be applied to remains, for example genetics. In some circumstances, the age of the remains or their severe damage (e.g., extreme subaerial weathering or thermal alteration), will not allow neither DNA extraction, nor any reliable measurements (57, 67). This information can save time and costs associated with running

biased analyses and can lead to different analytical techniques better suited to the specific samples.

Forensic taphonomy is not without limitations. Being a relatively new sub-discipline of forensic anthropology, it features several areas where research is quite scarce, or just emerging. Taphonomic investigations are limited for teeth in comparison to those available for bones. The damage acquired to bones and teeth deposited in aquatic and frozen environments is also understudied, alongside specific analytical techniques, such as DNA analysis, on bone and teeth that have been deposited into the environment for long periods, or that have undergone severe degradation, including thermal alteration and subaerial weathering. As more research is conducted in forensic taphonomy, the benefit of incorporating this subdiscipline into the workflow of forensic investigations is growing in relevance.

8. CONCLUSION

This review compiled and analysed the to-date information on the taphonomic processes that affect biomineralized tissues, bones, and teeth, across five distinct depositional environments: burial, subaerial exposure, aquatic environments, burnt and frozen remains. The unique taphonomic effects observed in each depositional environment can assist to increase the accuracy of the post-mortem history of human or non-human skeletal remains. As further research is conducted in this field, the benefit of incorporating forensic taphonomy knowledge into forensic investigations becomes increasingly apparent. The techniques of a forensic taphonomic investigation are not widely taught, even to forensic anthropologists. By enacting a multi-disciplinary approach to investigations through the inclusion of forensic

taphonomy, including the provision of training to current forensic anthropologists and investigators, a more thorough investigation can be undertaken, which may allow for better outcomes in the reconstruction of post-mortem histories, identification of remains and overall resolution of cases.

REFERENCES

1. Hu J, Liu X, Ma PX. Biomineralisation and Bone Regeneration. Second ed. Anthony Atala RL, James A. Thomson, Robert Nerem, editor: Academic Press; 2011.
2. Crichton RR. Biomineralisation. Second ed. Crichton RR, editor: Elsevier; 2012.
3. Senior KR. Bone and Muscle: Structure, Force, and Motion: The Rosen Publishing Group, Inc; 2010.
4. Rabiatul A, Fatihhi SJ, Harun MN, Kadir MRA, Ardiyansyah S, editors. A Narrative Review of Morphology of Cancellous Bone at Different Human Anatomy-Methods and Parameters. Applied Mechanics and Materials; 2015: Trans Tech Publ.
5. Pawlina W, Ross MH. Histology: a text and atlas: with correlated cell and molecular biology: Lippincott Williams & Wilkins; 2018.
6. Kono T, Sakae T, Nakada H, Kaneda T, Okada H. Confusion between Carbonate Apatite and Biological Apatite (Carbonated Hydroxyapatite) in Bone and Teeth. Minerals. 2022;12(2):170.
7. Houck MM, Siegel JA. Fundamentals of forensic science: Academic Press; 2009.
8. Byers SN. Introduction to forensic anthropology: Taylor & Francis; 2016.
9. Schotsmans EM, Márquez-Grant N, Forbes SL. Taphonomy of human remains: forensic analysis of the dead and the depositional environment: John Wiley & Sons; 2017.
10. Dupras TL. Forensic recovery of human remains: archaeological approaches: CRC Press; 2005.
11. Hayman J, Oxenham M. Human body decomposition: Academic Press; 2016.
12. Damann FE, Carter DO. Human decomposition ecology and postmortem microbiology. Manual of forensic taphonomy. 2013:37-49.
13. POKINES JT. Introduction: Collection of macroscopic osseous taphonomic data and the recognition of taphonomic suites of characteristics. Manual of forensic taphonomy: CRC Press; 2013. p. 16-33.
14. Pearson MP, Pearson MP. The archaeology of death and burial: Sutton Phoenix Mill, UK; 1999.
15. Pokines JT, Baker JE. Effects of burial environment on osseous remains. Manual of forensic taphonomy. 2013:73-114.
16. Buekenhout I, Vieira DN, Ferreira MT. Reliability of weathering in the estimation of the post-mortem interval of human remains buried in coffins. Australian Journal of Forensic Sciences. 2018;50(4):414-27.
17. Nicholson RA. Bone degradation, burial medium and species representation: debunking the myths, an experiment-based approach. Journal of Archaeological Science. 1996;23(4):513-33.
18. Egeland CP, Pickering TR. Cruel traces: Bone surface modifications and their relevance to forensic science. Wiley Interdisciplinary Reviews: Forensic Science. 2021;3(3):e1400.
19. Salesse K, Dufour E, Lebon M, Wurster C, Castex D, Bruzek J, et al. Variability of bone preservation in a confined environment: the case of the catacomb of Sts Peter and Marcellinus (Rome, Italy). Palaeogeography, Palaeoclimatology, Palaeoecology. 2014;416:43-54.
20. Kendall C, Eriksen AMH, Kontopoulos I, Collins MJ, Turner-Walker G. Diagenesis of archaeological bone and tooth. Palaeogeography, Palaeoclimatology, Palaeoecology. 2018;491:21-37.
21. Fisher JW. Bone surface modifications in zooarchaeology. Journal of Archaeological method and theory. 1995;2(1):7-68.
22. Child AM. Microbial taphonomy of archaeological bone. Studies in conservation. 1995;40(1):19-30.
23. Larson DO, Vass AA, Wise M. Advanced scientific methods and procedures in the forensic investigation of clandestine graves. Journal of Contemporary Criminal Justice. 2011;27(2):149-82.
24. Delannoy Y, Colard T, Le Garff E, Mesli V, Aubernon C, Penel G, et al. Effects of the environment on bone mass: a human taphonomic study. Legal Medicine. 2016;20:61-7.

25. Magni PA, Lawn J, Guareschi EE. A practical review of adipocere: Key findings, case studies and operational considerations from crime scene to autopsy. Journal of Forensic and Legal Medicine. 2021;78:102109.
26. Ross AH, Cunningham SL. Time-since-death and bone weathering in a tropical environment. Forensic Science International. 2011;204(1-3):126-33.
27. Madgwick R, Mulville J. Investigating variation in the prevalence of weathering in faunal assemblages in the UK: a multivariate statistical approach. International Journal of Osteoarchaeology. 2012;22(5):509-22.
28. Pokines JT, Zinni DP, Crowley K. Taphonomic patterning of cemetery remains received at the Office of the Chief Medical Examiner, Boston, Massachusetts. Journal of forensic sciences. 2016;61:S71-S81.
29. Guareschi E, Dadour IR, Magni PA. A taphonomic examination of inhumed and entombed remains in Parma cemeteries, Italy. Global Journal of Forensic Science & Medicine. 2019;1(4):1-8.
30. Pokines JT, Faillace K, Berger J, Pirtle D, Sharpe M, Curtis A, et al. The effects of repeated wet-dry cycles as a component of bone weathering. Journal of Archaeological Science: Reports. 2018;17:433-41.
31. Behrensmeyer AK. Taphonomic and ecologic information from bone weathering. Paleobiology. 1978;4(2):150-62.
32. Vietti LA. Quantifying bone weathering stages using the average roughness parameter Ra measured from 3D data. Surface Topography: Metrology and Properties. 2016;4(3):034006.
33. JUNOD CA, POKINES JT. Subaerial weathering. Manual of forensic taphonomy: CRC Press; 2013. p. 302-29.
34. Stokes S, Márquez-Grant N, Greenwood C. Establishing a minimum PMI for bone sun bleaching in a UK environment with a controlled desert-simulated comparison. International Journal of Legal Medicine. 2020;134(6):2297-306.
35. Miller GJ. A study of cuts, grooves, and other marks on recent and fossil bone: II. Weathering cracks, fractures, splinters, and other similar natural phenomena. World Anthropology. 1975:211-26.
36. Murphy L, Barnett BG, Holloway RG, Sheldon CM. An experiment to determine the effects of wet/dry cycling on certain common cultural materials. The final report of the National Reservoir Inundation Study Technical Report. 1981:8-1.
37. Trueman CN, Behrensmeyer AK, Tuross N, Weiner S. Mineralogical and compositional changes in bones exposed on soil surfaces in Amboseli National Park, Kenya: diagenetic mechanisms and the role of sediment pore fluids. Journal of Archaeological Science. 2004;31(6):721-39.
38. Guareschi EE, Nicholls PK, Evans NJ, Barham M, McDonald BJ, Magni PA, et al. Bone diagenesis in the marine environment-I: characterization and distribution of trace elements in terrestrial mammalian bones recovered from historic shipwrecks. International Journal of Osteoarchaeology.
39. Keenan SW. From bone to fossil: A review of the diagenesis of bioapatite. American Mineralogist. 2016;101(9):1943-51.
40. Fernández-Jalvo Y, Andrews P, Pesquero D, Smith C, Marín-Monfort D, Sánchez B, et al. Early bone diagenesis in temperate environments: Part I: Surface features and histology. Palaeogeography, Palaeoclimatology, Palaeoecology. 2010;288(1-4):62-81.
41. Symes SA, L'abbé EN, Pokines JT, Yuzwa T, Messer D, Stromquist A, et al. Thermal alteration to bone. Manual of forensic taphonomy: CRC Press; 2013. p. 382-417.
42. Schultz JJ, Warren MW, Krigbaum JS. Analysis of human cremains: gross and chemical methods. The analysis of burned human remains: Elsevier; 2008. p. 75-viii.
43. Ellingham ST, Thompson TJ, Islam M, Taylor G. Estimating temperature exposure of burnt bone—A methodological review. Science & Justice. 2015;55(3):181-8.
44. Shipman P, Foster G, Schoeninger M. Burnt bones and teeth: an experimental study of color, morphology, crystal structure and shrinkage. Journal of archaeological science. 1984;11(4):307-25.

45. Symes SA, Rainwater CW, Chapman EN, Gipson DR, Piper AL. Patterned thermal destruction of human remains in a forensic setting. The analysis of burned human remains: Elsevier; 2008. p. 15-vi.
46. Ubelaker DH. The forensic evaluation of burned skeletal remains: A synthesis. Forensic science international. 2009;183(1-3):1-5.
47. Shahack-Gross R, Bar-Yosef O, Weiner S. Black-coloured bones in Hayonim Cave, Israel: differentiating between burning and oxide staining. Journal of archaeological Science. 1997;24(5):439-46.
48. Dunlop JM. Traffic light discoloration in cremated bones. Medicine, Science and the Law. 1978;18(3):163-73.
49. Figueiredo M, Fernando A, Martins G, Freitas J, Judas F, Figueiredo H. Effect of the calcination temperature on the composition and microstructure of hydroxyapatite derived from human and animal bone. Ceramics international. 2010;36(8):2383-93.
50. Correia PM. Fire modification of bone: a review of the literature. Forensic taphonomy: The postmortem fate of human remains. 1997:275-93.
51. Snoeck C, Lee-Thorp J, Schulting R. From bone to ash: Compositional and structural changes in burned modern and archaeological bone. Palaeogeography, palaeoclimatology, palaeoecology. 2014;416:55-68.
52. Harbeck M, Schleuder R, Schneider J, Wiechmann I, Schmahl WW, Grupe G. Research potential and limitations of trace analyses of cremated remains. Forensic science international. 2011;204(1-3):191-200.
53. Mckinnon M, Henneberg M, Simpson E, Higgins D. A comparison of crystal structure in fresh, burned and archaic bone–Implications for forensic sampling. Forensic Science International. 2020;313:110328.
54. Bromage TG, Goldman HM, McFarlin SC, Warshaw J, Boyde A, Riggs CM. Circularly polarized light standards for investigations of collagen fiber orientation in bone. The Anatomical Record Part B: The New Anatomist: An Official Publication of the American Association of Anatomists. 2003;274(1):157-68.
55. Collins MJ, Nielsen–Marsh CM, Hiller J, Smith C, Roberts J, Prigodich R, et al. The survival of organic matter in bone: a review. Archaeometry. 2002;44(3):383-94.
56. Jellinghaus K, Urban PK, Hachmann C, Bohnert M, Hotz G, Rosendahl W, et al. Collagen degradation as a possibility to determine the post-mortem interval (PMI) of human bones in a forensic context–A survey. Legal Medicine. 2019;36:96-102.
57. Higgins D, Rohrlach AB, Kaidonis J, Townsend G, Austin JJ. Differential nuclear and mitochondrial DNA preservation in post-mortem teeth with implications for forensic and ancient DNA studies. PloS one. 2015;10(5):e0126935.
58. Squires KE, Thompson TJ, Islam M, Chamberlain A. The application of histomorphometry and Fourier Transform Infrared Spectroscopy to the analysis of early Anglo-Saxon burned bone. Journal of Archaeological Science. 2011;38(9):2399-409.
59. Castillo RF, Ubelaker DH, Acosta JAL, de la Fuente GAC. Effects of temperature on bone tissue. Histological study of the changes in the bone matrix. Forensic science international. 2013;226(1-3):33-7.
60. Piga G, Thompson TJ, Malgosa A, Enzo S. The potential of X-ray diffraction in the analysis of burned remains from forensic contexts. Journal of Forensic Sciences. 2009;54(3):534-9.
61. Bonucci E, Graziani G. Comparative thermogravimetric, x-ray diffraction and electron microscope investigations of burnt bones from recent, ancient and prehistoric age. Atti della Accademia Nazionale dei Lincei Classe di Scienze Fisiche, Matematiche e Naturali Rendiconti. 1975;59:517-32.
62. Neson R. A microscopic comparison of fresh and burned bone. Journal of Forensic Science. 1992;37(4):1055-60.

63. Herrmann B. Über die Abhängigkeit der Schrumpfung vom Mineralgehalt bei experimentell verbrannten Knochen. Anthropologischer Anzeiger. 1977:7-12.
64. Herrmann B. On histological investigations of cremated human remains. Journal of Human Evolution. 1977;6(2):101-3.
65. Herrmann B. Neuere Ergebnisse zur Beurteilung menschlicher Brandknochen. Zeitschrift für Rechtsmedizin. 1976;77(3):191-200.
66. Walker PL, Miller KW, Richman R. Time, temperature, and oxygen availability: an experimental study of the effects of environmental conditions on the color and organic content of cremated bone. The analysis of burned human remains: Elsevier; 2008. p. 129-xi.
67. Rubio L, Sioli JM, Gaitán MJ, Martin-de-Las-Heras S. Dental color measurement to predict DNA concentration in incinerated teeth for human identification. PloS one. 2018;13(4):e0196305.
68. Schmidt CW. Burned human teeth. The analysis of burned human remains: Elsevier; 2015. p. 61-81.
69. Beach JJ, Passalacqua NV, Chapman EN. Heat-related changes in tooth color: temperature versus duration of exposure. The analysis of burned human remains: Elsevier; 2008. p. 137-xi.
70. Wilson DF, Massey W. Scanning electron microscopy of incinerated teeth. The American Journal of Forensic Medicine and Pathology. 1987;8(1):32-8.
71. Garriga JA, Ubelaker DH, Zapico SC. Evaluation of macroscopic changes and the efficiency of DNA profiling from burnt teeth. Science & Justice. 2016;56(6):437-42.
72. Evans T. Fluvial taphonomy. Manual of forensic taphonomy: CRC Press; 2013. p. 130-57.
73. Higgs ND, Pokines JT. Marine environmental alterations to bone: CRC Press Boca Raton, FL; 2014.
74. Pokines JT, Menschel M, Mills S, Janowiak E, Satish R, Kincer C. Experimental Formation of Marine Abrasion on Bone and the Forensic Postmortem Submergence Interval. Forensic Anthropology. 2020;3(4):175.
75. Guareschi EE, Tobe SS, Nicholls PK, Magni PA. Taphonomy and Diagenesis of Human Bone in Underwater Archaeology: A Review of the Current Status and the Proposal of Post-Mortem Submersion Interval (PMSI) as a Potential Forensic Application. Journal of Maritime Archaeology. 2021;16(1):57-75.
76. Pokines JT, Higgs N. Macroscopic taphonomic alterations to human bone recovered from marine environments. J Forensic Identif. 2015;65(6):953-84.
77. Haglund WD. Disappearance of soft tissue and the disarticulation of human remains from aqueous environments. Journal of Forensic Science. 1993;38(4):806-15.
78. Christensen AM, Myers SW. Macroscopic observations of the effects of varying fresh water pH on bone. Journal of forensic sciences. 2011;56(2):475-9.
79. Guareschi EE, Haig DW, Tobe SS, Nicholls PK, Magni PA. Foraminifera—A new find in the microtaphonomical characterization of bones from marine archaeological excavations. International Journal of Osteoarchaeology. 2021;31(6):1270-5.
80. Pesquero MD, Bell LS, Fernández-Jalvo Y. Skeletal modification by microorganisms and their environments. Historical Biology. 2018;30(6):882-93.
81. Belaústegui Z, de Gibert JM, Domènech R, Muñiz F, Martinell J. Clavate borings in a Miocene cetacean skeleton from Tarragona (NE Spain) and the fossil record of marine bone bioerosion. Palaeogeography, Palaeoclimatology, Palaeoecology. 2012;323:68-74.
82. Zazzo A, Lécuyer C, Mariotti A. Experimentally-controlled carbon and oxygen isotope exchange between bioapatites and water under inorganic and microbially-mediated conditions. Geochimica et Cosmochimica Acta. 2004;68(1):1-12.
83. Tütken T, Vennemann T, Pfretzschner H-U. Early diagenesis of bone and tooth apatite in fluvial and marine settings: constraints from combined oxygen isotope, nitrogen and REE analysis. Palaeogeography, Palaeoclimatology, Palaeoecology. 2008;266(3-4):254-68.
84. Trueman C, Benton MJ, Palmer M. Geochemical taphonomy of shallow marine vertebrate assemblages. Palaeogeography, Palaeoclimatology, Palaeoecology. 2003;197(3-4):151-69.

85. Pilloud MA, Megyesi MS, Truffer M, Congram D. The taphonomy of human remains in a glacial environment. Forensic Science International. 2016;261:161. e1-. e8.
86. Turpin C. The Micro-Taphonomy of Cold: Differential Microcracking in Response to Experimental Cold-Stresses. Journal of forensic sciences. 2017;62(5):1134-9.
87. Maschenko E, Potapova O, Heintzman P, Kapp J, Shapiro B, Protopopov A, et al. Morphology, Individual Age, DNA and Sex of the Yuka Mammoth (Mammuthus primigenius) from Northern Yakutia, Russia. Paleontological Journal. 2021;55(11):1230-59.
88. Roberts LG, Dabbs GR. A Taphonomic Study Exploring the Differences in Decomposition Rate and Manner between Frozen and Never Frozen Domestic Pigs (S us scrofa). Journal of forensic sciences. 2015;60(3):588-94.
89. Maschenko EN, Potapova OR, Vershinina A, Shapiro B, Streletskaya ID, Vasiliev AA, et al. The Zhenya Mammoth (Mammuthus primigenius (Blum.)): Taphonomy, geology, age, morphology and ancient DNA of a 48,000 year old frozen mummy from western Taimyr, Russia. Quaternary International. 2017;445:104-34.
90. Hale AR, Ross AH. The impact of freezing on bone mineral density: implications for forensic research. Journal of forensic sciences. 2017;62(2):399-404.
91. Tersigni MA. Frozen human bone: a microscopic investigation. Journal of forensic sciences. 2007;52(1):16-20.
92. Pokines JT, L'Abbe EN, Symes SA. Manual of forensic taphonomy: CRC Press; 2021.

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Part Two

Manuscript

Back in the cycle:

a review of the taphonomy of biomineralised tissues

Abstract

Biomineralized tissues, such as bone and teeth, are composed of an organic and an inorganic portion. After death, biomineralized tissues are often the last remaining structures of a living vertebrate organism. The depositional environment, and the events that have affected them in the post-mortem period, can provide a valuable insight into the taphonomic processes of any biomineralized tissues recovered in an excavation/investigation. Forensic taphonomy, being a relatively new sub-discipline of forensic anthropology, studies the post-mortem modifications of remains in relation to a variety of common taphonomic agents, physical, chemical, or biological. Several research and case studies have considered the taphonomic effects observed in single depositional environments. This review presents a summary and a compilation of the to-date information on the effects generated on bones and teeth by the taphonomic agents present across the most common depositional environments: burial, subaerial exposure, aquatic environments, and extreme temperatures (burnt, frozen). This review also highlights the several areas in which this discipline is currently limited, including the taphonomic processes in frozen and aquatic environments, and of the post-mortem alterations of teeth. Each depositional environment produces a range of characteristic taphonomic effects, which may be used to generate a more accurate description of the post-mortem histories of remains. By providing training in forensic taphonomy investigative techniques, and incorporating them into forensic investigations, more precise information may be gathered, potentially leading to faster turnaround times and case resolutions. The information presented in this review will be used to assist the forensic community and may be vital to future research efforts and forensic investigations.

Keywords: forensic, anthropology, decomposition, bone, teeth, diagenesis, osteoarchaeology

Table of Contents

List of Abbreviations

BA	Bioapatite
DNA	Deoxyribonucleic acid
GI	Gastrointestinal
HA	Hydroxyapatite
IR	Infra-red
mtDNA	Mitochondrial DNA
PMI	Post-mortem interval
PMSI	Post-mortem submersion interval
REEs	Rare earth elements
SEM	Scanning electron microscope/microscopy
UV	Ultra-violet
µm	Micrometre/micron
CO_2	Carbon dioxide
°C	Degrees Celsius
Wt.%	Percentage weight

1. INTRODUCTION

Biomineralization is a complex process that results in the formation of structured organic-inorganic tissues, like bone and teeth, by depositing inorganic mineral crystals into the organic matrix of living vertebrate organisms. These tissues are formed during the early stages of an organism's foetal development, and continue to be replaced throughout life in response to stimuli, such as growth or damage (1, 2). The two different types of biomineralized tissues are bones and teeth.

Bone is one of the hardest substances in a vertebrates' body, including humans' (3) and performs four main functions: motion at the joints by acting as levers powered by attached muscles, structural support, protection of vital organs, and centres of growth as the body transitions from infancy to adulthood (7). It is typically characterised as a specialised form of connective tissue, consisting of an organic component (mostly type I collagen) and an inorganic (or mineral) component in the form of crystals of bioapatite (BA), a type of hydroxyapatite (HA) containing carbonic acid (5) (6). Hydroxyapatite consists primarily of calcium and phosphate (46). The outer portion of bone is made of a dense, compact bone, called cortical. This tissue often appears smooth due to the linear arrangement of collagen fibrils, parallel to the longer axis of the bone (8). A second type of bone tissue is found in the epiphyses of long bones, within vertebral bodies and ribs, and within all bones of the hands and feet, among a few other anatomical locations (8). This tissue, known as trabecular, spongy, or cancellous bone, is arranged in a porous, spiderweb pattern (3), providing bones with support without adding excess weight (8).

Teeth are made of inorganic and organic components similar to bone, but with a significantly higher mineral content (9). Teeth are formed during foetal development and are anchored in the alveolar processes of the mandible and maxilla ("jaw bones"). Humans have two sets of teeth: deciduous teeth (also known as "baby teeth") that fall out and are replaced by permanent teeth (also known as "adult teeth") as they grow older (5). In humans, there are four morphological tooth types: incisors, canines, premolars, and molars, which are all made up of enamel, dentin, and cementum (10). The crown, or visible portion of the tooth, is covered in enamel, which is 99% mineral. Dentin is 75% mineral and lies beneath the enamel, making up most of the tooth. Cementum, which is about 65% mineral, helps to keep the tooth's root, or hidden portion, connected to the jaw (8). The primary function of teeth is to facilitate mechanical digestion of food by mastication (7). The higher mineral content of teeth compared to that of bone accounts for their remarkable preservation after death and decomposition, allowing for post-mortem identification and taphonomic reconstructions (8). The modifications that occur to an organism after death, as the organic components pass from the biosphere into the lithosphere, are studied in the branch of science known as taphonomy (8, 13). Interest was first gained in this area from geologists and palaeontologists, who studied fossil preservation (13). Taphonomy now applies to processes altering both modern bone and ancient remains, often overlapping with other disciplines, such as anthropology and palaeontology, in terms of analytical techniques and interpretations of findings (13). Forensic taphonomy, in particular, is a sub-discipline of forensic anthropology that deals with the post-mortem modifications on human remains and any associated evidence, such as clothing, in relation to well-recognised taphonomic agents, such as animals, bacteria, plants, temperature, humidity and chemical alterations (10). These taphonomic agents vary greatly across different natural or artificial environments, from subaerial

exposure to burials or submerged conditions. Understanding the different taphonomic agents common to each depositional environment allows for a more detailed analysis and interpretation of the processes that occurred at the time of death (perimortem) and after death (post-mortem). As the strongest substances in the human body, biomineralized tissues, are often all that is left when all other tissues have decomposed (13). Therefore, determining which processes affected these tissues after death can aid forensic investigators in estimating the circumstances surrounding death, as well as the time since death, also known as the post-mortem interval (PMI). These investigations add clues to the identification of individual/s, which is the starting point of any forensic investigation on human skeletal remains.

Several research and case studies have considered the taphonomic effects observed in single depositional environments. This review presents a summary and compilation of the to-date information surrounding the taphonomic agents present across five natural depositional environments (burial, subaerial exposure, aquatic environments, burnt and frozen), and the effects generated on biomineralized tissues, both bones and teeth. Remains located in artificial environments - such as suitcases and freezers - or affected by other intrinsic/extrinsic factors such as embalming prior to burial, can demonstrate significantly different rates of decomposition and taphonomic alteration, making forensic analyses much more complex, so will not be considered in this review.

This review also highlights the several areas in which this discipline is currently limited, including the taphonomic processes in frozen and aquatic environments, and of the post-mortem alterations of teeth. The information gathered can then be used to assist and inform the forensic community in research and crime scene investigative techniques.

2. METHODS

For this review, references were obtained by searching the available literature, considering research performed via Murdoch University Library databases between January – June 2022. To find information regarding the taphonomic processes of biomineralized tissues in varying depositional environments, the following key words were utilised alone or in combination when searching for sources: 'bone/s', 'teeth', 'taphonomy', 'buried', 'bioerosion', 'frozen', 'burnt', 'exposure', 'subaerial', 'weathering', 'decomposition', 'aquatic'.

3. RESULTS

TAPHONOMY IN BURIAL ENVIRONMENTS

Burial environments involve the disposal of the deceased individual through placement of their remains into a grave, either a clandestine hole or pit, or a formal burial in a coffin. This process is also referred to as interment, or inhumation, indicating that the remains are placed at a subterranean level (14). The factors that affect the taphonomic changes in such environments are a combination of the way the bodies have been buried (i.e., type of coffin, if any), geological/physical/chemical characteristics of the soil, and micro/macro-organisms present in the soil (bioerosion). Overall, the taphonomic changes on bone have been studied more extensively than those on teeth.

Coffin wear and warping. When bodies are deposited in coffins signs of wear and warping can be observed on bony elements in constant and prolonged contact with the coffin floor, such as the occipital bone, posterior pelvis, calcaneus, and scapular and vertebral spines (15, 16, 28). This wear often presents as a localised form of cortical erosion, with a flattened or sheared appearance (15, 28). Warping can occur through similar mechanisms as coffin wear

and is exacerbated by the pooling of water on the coffin floor. This can degrade the bone matrix making it more prone to deformation and damage. The coffin environment can also become compromised over time, leading to collapse and the infiltration of soil. This compression can also damage bones directly, especially the cranium due to its 'hollow' structure (15, 28).

Soil characteristics. Human remains deposited directly into the soil zone, or first within a coffin, are directly affected by the composition and pH of the surrounding soil, which can result in altered decomposition rates, discolouration, and severe damage from acidic soil corrosion. Soils with a fine composition, such as clay soils, are often better at preserving remains, as the small particles will retain higher moisture levels, restricting the proliferation of aerobic microorganisms, and lessening the risk of external damage of bones and teeth through sediment abrasion. Soils with larger particles, such as sandy soils, often have lower moisture levels and better air flow, allowing for faster desiccation of soft tissues, and leading to higher incidences of damage to the cortical surfaces of bones, as the larger particles act as abrasive agents (9, 18). The soil composition can also result in discolouration of the remains and include mineral discolouration from copper (Cu) and iron (Fe) particles within the soil, a dark brown discolouration from tannin compounds, and a reddish staining from highly oxidised red clay soils. Additionally, some moulds present within the soil will colonise bones to produce a pink or mauve staining (15).

The pH level of soil has a direct effect on the decomposition rate and degree of preservation of tissues. Neutral and slightly alkaline soils tend to favour bone preservation, due to the relative insolubility of BA at these pH levels (9, 17). Some bacterial action may result in bone damage and discolouration, though the damage is minimal compared to highly acidic soils

(22). Low pH soils have been shown to be the most destructive to biomineralized tissues, due to fungal action and acidic soil corrosion. Demineralization of HA is promoted in low pH conditions, leading to reduced porosity and structural integrity of bone (15, 22). Some unique environments, such as acidic, waterlogged bogs, can result in the dissolution of biomineralized tissues and the mummification of soft tissues (15).

Bioerosion. Human remains deposited into a burial environment are frequently colonized by organisms like scavengers, bacteria, fungi, and plant roots, which cause characteristic damage termed bioerosion. Immediately after death, the physiological homeostatic mechanisms within the body are rapidly disrupted until complete cessation, this leads to the transmigration of enteric bacteria from the gastrointestinal (GI) system throughout the rest of the body (9), and is followed by a series of known stages of decomposition, such as bloating, decay (active and advanced) and skeletonization. Once all the soft tissues have been removed, specific classes of bacteria, known as collagenases, begin to feed on the bone collagen. The saprophytic action of these bacteria, which function at an optimal pH range of 7.3-7.4 (9), results in the formation of penetrating tunnels and destructive foci throughout bone (17, 18). The gradual destruction of bone occurs through thinning, disintegration, and cortical exfoliation. The optimal pH range of these bacteria mean they are most active in neutral soils and are commonly found on clandestine remains buried within these soils (9).

Fungi consist of long strings, called hyphae, that allow them to attach to substrates to grow and feed. They frequently grow in large groups, which can increase any damage they cause to a substrate such as bone (15). Like bacterial collagenases, fungi are saprophytic and release acids to dissolve the cortical layer of bone while extracting nutrients (17, 18). This acidic attack can lead to long term damage presenting as cortical exfoliation of bone (15). The hyphae can

also penetrate bone, leaving behind characteristic tunnelling (17). They typically favour more acidic soils, and are inhibited in waterlogged soils due to the anoxic conditions, which aren't compatible with aerobic fungi (17).

The phosphate and nitrogen content of bone makes it an appealing food source for many other plants. Root etching, appearing as an irregular pattern of thin, shallow lines, can be identified on bone as the result of plant roots adhering to the surface or penetrating throughout (15, 21). Roots secrete several compounds, including humic and citric acids, to aid in mineral uptake, however this action also destroys the area of bone they are in contact with (21). Root growth can also damage bone when its size becomes larger than the space it is occupying, leading to cracking from the inside out (15).

Teeth in burial environments. Teeth are often better preserved than bones in burial environments, due to their higher mineral content; however, research surrounding the taphonomic histories of teeth in burial environments is severely limited. Remains deposited directly into the soil zone will be exposed to the effects of taphonomic agents such as soil pH and bioerosion faster than those within a coffin environment, as the coffin will limit access to these agents. Once the coffin structure becomes compromised, the remains will be exposed directly to the surrounding soil and its encompassing taphonomic agents, resulting in similar damage as those directly deposited into the soil zone; therefore, you could have two sets of remains interred at the same time, with one directly deposited into the soil zone, and the other within a coffin environment, resulting in differing taphonomic histories. One study demonstrated that teeth can be affected by acidic soil pH levels similar to the effects seen in bone. The increased enamel porosity from acidic soil corrosion can lead to discolouration and staining from the immediate soil environment (93). Another study of teeth found in a coffin

environment from the Bronze Age exhibited excellent preservation of the enamel, along with other proteinaceous material such as fingernails and hair, whilst all other skeletal material had disintegrated (20).

TAPHONOMY IN SUBAERIAL ENVIRONMENTS

The deposition of remains at the ground surface, exposed to the natural elements without the protection of a burial, characterises subaerial environments. In that condition, remains gradually undergo decomposition, separation and destruction of both organic and inorganic components (30, 31). Subaerial environments can encompass a variety of environments, including desert, tropical and frozen landscapes, with their associated microenvironments (temperature and humidity fluctuations) playing a direct role in the rate of decomposition and associated taphonomic alterations seen on the remains. This review will focus largely on subaerial environments in temperate climates, which have been more extensively researched, with only brief mention of the effects seen in other climates. Whilst subaerial environments may become secondary depositional environments at some point in the taphonomic history of the remains, e.g., when a bone washes up on the shoreline and is taken into the forest by a scavenger, only research pertaining to the taphonomic alterations seen on remains where a subaerial environment was their primary location of deposition will be considered below.

Weathering. Subaerial weathering can be defined as a taphonomic process whereby the biomineralized tissues decay and break down over time. This is due to the prolonged exposure to various taphonomic agents that work mechanically, biologically, and chemically to separate

and destroy their organic and inorganic components (27, 30, 32). The weathering begins once the decomposition process has removed most, or all, soft tissues (8). Damage is characterised as cortical exfoliation, cracking and flaking of the surface layers, bleaching, loss of moisture and total disintegration (27, 32, 33). The rate of weathering is dependent on the microenvironment the remains are deposited into, which dictates amount of vegetation coverage, degree of sun exposure, humidity, and temperature fluctuations. If the deposition environment is known, degrees of weathering damage can be broken down into five stages (31). As rates of weathering are linked to time since death, they can cautiously be used as a proxy for the estimation of the PMI in forensic cases, however the rate of decomposition of soft tissues will need to be considered, as bone weathering will not begin until soft tissues persist (30).

UV exposure. Prolonged exposure of bones to UV light and heat from the sun is the primary cause of weathering in subaerial environments, resulting in characteristic damage in the form of bleaching and cracking. Early in the weathering process, UV rays break down the connections between molecules inside the bone matrix, resulting in photodegradation, or colour loss (33, 34). One study showed bleaching to occur quicker in summer than winter, with bones deposited in shaded areas not bleaching at all (34). Cracking and delamination also accompany bleaching, as the loss of moisture from constant UV exposure results in the shrinkage of the outermost cortical layers of bone. This shrinkage puts tension on the cortical surface until it cracks, often flaking away in layers until the bone has completely disintegrated (8, 33). The degree of UV exposure, dependent on the geographical location and microenvironment the remains are in, will directly determine the rate of crack propagation

and bleaching on exposed bones, with some remains showing little degradation after 30 years, while others may be severely eroded in as little as six years (27).

Freeze-thaw and wet-dry cycles. Extreme fluctuations in temperatures, such as those below freezing (0°C/32°F), to more moderate temperatures in the range of 20-25°C (68-77°F) may lead to damage in the form of cracking, flaking, and warping, with such effects exacerbated when moisture is present within the bone, due to the expansion water molecules undergo upon freezing, which exert a large destructive force upon the surrounding structures (33). Some microenvironments experience frequent freeze-thaw cycles, like those found in locations like Iceland and Patagonia, which can directly affect the degree of bone preservation. Bones in these environments tend to degrade faster than those deposited into more constant environments, such as subtropical or tundra regions.

Any water available in the bone tissue, including water that seeps into available spaces from the surrounding environment, will initially freeze, expanding as ice crystals are formed (33). This expansion can exert large forces upon the bones, which can lead to the formation of new cracks, or the expansion of existing cracks and pore spaces. When the ice melts, more room is available within the bone for water to occupy, which can then re-freeze in the next freezing cycle, resulting in a continuous cycle of destruction (33).

Whilst freeze-thaw cycles tend to produce damage to the interior of bones, wet-dry cycles more frequently affect the outer surfaces. When bone is exposed to moisture in the form of precipitation or contact with groundwater in the soil, the tissues will partially rehydrate, until they once again become dried out by daily UV exposure. The amount of moisture and UV exposure remains are exposed to depends solely on the microenvironment of the

depositional location (33). Repeated wet-dry cycles over time can generate damage in the form of cracking, flaking, and warping. As moisture is evaporated from bone, including the leaching of fat from the medullary cavities, the bone tissue shrinks, resulting in the cracking and flaking of cortical layers (33). Rehydration of the bones can then result in further damage as the bone dries out again (30, 33). Contact with groundwater can also result in the formation of authigenic crystals within the pore spaces of bone as groundwater evaporates (33, 37). These crystals can alter the physical and microscopic structure of the bone, weakening the bone matrix through recrystallisation of the HA, and resulting in a less homogeneous overall composition (33). Chemical and rare earth elements (REEs) can also be taken up into the bone structure, resulting in a trace element composition very similar to that of surrounding pore water; this can suggest a geographical marker of the depositional location of the remains (37) (38).

Bioerosion and soil pH. In a subaerial environment, bones and teeth that are in contact with the soil can be affected by bioerosion - from algae, fungi, moss, and lichen - as well as acidic soil corrosion from low pH soils. Algae growth is common on exposed bones, particularly on the upper surface, where they use the sun for photosynthesis. Evidence of algal growth involves the formation of microtunnels, which can appear similar to damage caused by other bone-boring microorganisms but will be more superficial and homogenous. Algae are not saprophytic like bacteria and fungi, so will penetrate bone using Haversian canals (40). Fungal damage will appear as microscopic focal damage in the form of characteristic Wedl-tunnelling; this damage is often more apparent on completely buried bones, though is not uncommon for bones deposited subaerially (33, 40). Mosses use bone as a substrate for growth, penetrating the bone through Haversian canals in a similar manner to fungi (33).

Damage presents in the form of staining, microtunnelling, small pitting, and edge rounding of surfaces. Moss growth can also break thinner bones, such as immature cortical bone or trabecular bone (40). The damage to bone caused by lichen is similar to that of algae and moss, with frequent shallow microtunnelling observed, alongside holes, fissures and cracks within the cortical bone surface (40).

Characteristic macroscopic damage is evident on bones that are in contact with acidic soils and presents as scooping out of articular ends and cortical layer degradation. Histologically, this damage appears as thin microtunnelling, a reduction in overall bone density, and the degradation of the cortical layer, exposing osteons and interstitial lamellae (40). Damage to bones from prolonged contact with acidic soil has been likened to that of scanning electron microscopy (SEM) images of bone digested by gastric stomach acids (40).

Teeth in subaerial environments. There is comparatively little information on the taphonomic alterations affecting teeth in a subaerial depositional environment. Some experimental studies have observed the propagation of cracks in the pulp cavity terminating at the dentin-enamel junction. This is in direct contrast to cracks formed on teeth from trauma, which travel from the outside surface inwards (8). Many variables can affect the rate of weathering on teeth however, such as the degree of enamel wear, stage of eruption, and overall morphology at death (31).

TAPHONOMY OF BURNT REMAINS

Burnt remains have undergone thermal alteration through combustion for varying time durations because of several possible events, such as motor vehicle accidents, plane crashes, house fires and natural disasters. The nature of the thermal alteration, such as the

decomposition stage of the body at the time of burning and materials used to create the heat source, can affect the resulting damage, including changes in the overall burn pattern, crystal structure, histological appearance, chemical and biological characteristics, and any colour changes.

Physical appearance. As biomineralised tissues undergo thermal alteration, several visible changes will occur, such as cracking, flaking, carbonisation, and calcination, depending on the highest thermal alteration stage the bone reached. Each stage delineates characteristic damage markers and other changes that occur to biomineralised tissues within a certain temperature range. There are currently four identified stages: dehydration, decomposition, inversion, and fusion.

Within the dehydration stage, bone may present several common types of fracture consistent with burning. Longitudinal fractures are the most common, typically occurring in long bones, due to rapid shrinkage and loss of moisture. These fractures are often parallel with histological structures such as Haversian canals and may run the entire length of the diaphysis (41, 45). Transverse factures run perpendicular to longitudinal fractures, transecting Haversian canals, as the bone tissue shrinks incrementally when soft tissues are vaporised. Patina fractures imitate those seen in old chinaware and are most commonly observed superficially on flat postcranial bones like the ribs and sternum. These fractures are hypothesised to form in response to large areas of bone being heated homogenously, leading to simultaneous shrinkage (41, 45). The majority of collagen is lost during the decomposition stage, resulting in shrinkage and carbonisation (43, 44). As this organic component is lost, oxygen and hydrogen molecules are liberated from the matrix, leaving behind carbon and the inorganic component of BA, which gives the bone a black colouration (41). Inversion involves

the devolatilization of all remaining organic components, and the liberation of carbon from the matrix by combining with oxygen to form CO_2. The bone structure becomes extremely brittle and will present a white or light grey colouration (41, 43, 44). Fusion occurs when bones are cremated at extreme temperatures of approximately 900°C and above, resulting in the fusing of inorganic mineral salts to form a homogenous matrix (41).

Colour changes. As bones and teeth are subjected to increasing duration and temperature of heat exposure, changes in the colour of the visible tissues will occur, reflecting the proportion of organic to inorganic components remaining, or as a result of contact with certain metals during combustion (43, 46, 47). Copper will produce a pink colour on bone, whilst iron and zinc will produce a green and yellow staining, respectively (48). Manganese or iron oxides can stain bone black, so the immediate environment needs to be taken into consideration when determining whether bones are black as a result of staining or carbonisation. Analysis of infrared (IR) spectrums of these compounds can distinguish between carbonised and black-stained bones (47). Bones and teeth will progress through identifiable colour changes, depending on the temperature and/or duration of combustion. Initially bones will be a natural off-white colour, progressing to brown and black as carbonisation occurs, then blue-grey and white as the bone becomes calcined (17, 43, 44, 46, 49-53, 58-65). Teeth go through almost identical colour changes, though vary slightly as enamel and dentin contain different mineral compositions. Enamel begins at a natural white colour, becoming a pale brown then black as carbonisation occurs, to light grey when calcined. Dentin, with a higher organic content, will progress through colour changes more rapidly than enamel, from natural white through to black when carbonised. No further colour changes have been identified for enamel and dentin, indicating more research is required in this area (44, 67, 69-71).

Histology and crystallinity. Morphological changes in the microstructure of bone and teeth that occur due to intense and prolonged heat exposure, can be identified at the microscopic level through histology. Histological analysis of biomineralized tissues occurs on a micron scale and can assess differences in microstructures before and after heat-alteration, including bone porosity, microcracking, and a gradual reduction or complete loss of structures such as Haversian systems, Volkmann's canals, lamellae, and osteocyte lacunae. It has been demonstrated that with exposure to prolonged heat and increasing temperatures, these microstructures gradually change shape and decrease in size, leaving behind a uniformly granular matrix with little similarity to fresh bone and teeth tissue (43, 49, 50).

Crystallinity refers to multiple characteristics of BA crystals within bone and teeth. Fresh bone typically has low crystallinity and small crystal sizes, and the BA component is highly reactive in this form (51). As thermal alteration progresses, the crystal sizes increase and take on new shapes and a more regular arrangement, and the reactivity of BA decreases (50, 51). To measure changes in crystallinity, techniques such as IR spectroscopy and X-ray diffraction are commonly utilised (51, 52).

Chemical and biological changes. The chemical structure of burnt bones and teeth can be analysed alongside the physical appearance to provide a more detailed understanding of the degree of thermal alteration that has occurred. When a certain temperature is reached, the crystals begin to coalesce as fusion and mineral sintering occur, eventually resulting in the formation of small, rounded granular structures. This process will occur until the complete cremation of bone and teeth, when only ash remains (44, 50, 53). Stable isotope analysis can be performed on bone and teeth tissues to provide information about the history of an

individual, including their geographical origins and lifestyle. Both light isotopes (C, N) and heavy isotopes (^{18}O, $\delta^{13}C$, $\delta^{15}C$) can be analysed, though results are shown to be reliable only to a temperature of approximately 200°C. This is typically when bone reaches the stage of cremation where it begins to turn brown in colour, but has not yet carbonised (52).

Increased thermal exposure of bones and teeth can begin to alter the biological makeup of the tissues, degrading DNA material to a point where extraction methods are no longer viable and resulting in a reduction of overall collagen content. With increasing temperatures, collagen is gradually lost from 11 wt.% when fresh to 0.1 wt.% at temperatures >900°C (50, 52). The analysis of collagen content is achieved through histology, by measuring birefringence in polarised light or collagen content by specific staining techniques in ordinary light microscopy (54-56). A decrease in birefringence or specifically stained areas in histological slides indicate a reduction in collagen content, which gradually becomes absent once all collagen has been depleted (52). Some studies were able to successfully extract and amplify readable mitochondrial DNA (mtDNA) from samples that had been subjected to temperatures of up to 700°C, though the quality of results was significantly reduced (57). Whether or not a sample will be able to produce sufficient DNA results can only be assessed through histological and birefringence analysis (52).

Composition of remains. The degree of heat-alteration seen is greater in bones covered by small amounts of tissue, such as the frontal bone of the cranium, than in bones covered more extensively, such as the femur, and can also be affected by the decomposition stage of a body, and bodies with higher fat percentages (41, 45). When fresh remains are exposed to extreme heat, they assume the pugilistic posture. The flexor muscles contract as the fire rapidly dehydrates and shrinks them, resulting in severe flexion of the postcranial elements, most

notably in the upper and lower appendages (41, 45, 46). The flexed posture exposes some anatomical regions more than others. This phenomenon affects the wrists, elbows, and knees first, resulting in differential burning, with the upper, more exposed areas exhibiting increased damage. (41, 45). It is not uncommon to see the entire spectrum of heat modifications on a single bone, from fresh bone to charred and calcined sections due to the variations in tissue depth and body posture during burning. Due to the extreme heat rapidly removing all moisture and collagen, as well as the stresses generated when the muscles, ligaments, and tendons shrink away from their attachment points, fleshed bones will display extreme shrinkage, warping, and fracturing (41, 46).

A body that has been slightly decomposed will behave similarly to a fresh body, with the same fracture patterns and burn morphologies. A more thoroughly decomposed body, on the other hand, can show abnormal burn patterns. The disappearance through decomposition of muscles, skin, tendons, and ligaments means the traditional pugilistic posture may not have been assumed. This results in burned bone pieces that would otherwise have been shielded throughout the creation of the pugilistic posture (41).

Bones that are not encased in soft tissues burn with a more uniform pattern. They frequently reach calcination faster than fleshed bones, with less cracking and warping since much of the moisture content has already evaporated prior to the burning event. Longitudinal fractures remain common when the bone dries and the collagen content is gradually reduced, however many of the other effects seen in fleshed bones are not visible (41, 46).

Teeth from burnt remains. Not as much research has been conducted into the effects that varying temperatures have on the taphonomy of teeth in comparison to bone. In the forensic context, teeth can be used to identify an individual when other means of identification (e.g.,

visual) are not viable. This is often the case when remains are burnt beyond recognition, or when only bones and teeth remain (68). The teeth enamel and dentin have a lower organic component, and a different microstructure than bone (e.g., rods instead of osteons), which contributes to their post-mortem survivability in the environment (69). Despite this difference, teeth progress through the same general stages of heat-alteration as bone, including carbonisation and calcination (41). Dentin will often exhibit these changes faster than enamel, due to their differing levels of mineral content, meaning one may show signs of carbonisation when the other appears unaltered (68). The fracture patterns identified in heat-altered bone are also evident in burnt teeth specimens. Roots will often display transverse and step fractures, while enamel may display longitudinal cracks along lingual, central and labial grooves (68). Similar colour changes occur in enamel and dentin at varying temperatures as those seen in bone. They will begin as a pale white-yellow colour when fresh, progressing to a pale brown and dark reddish brown, respectively, as temperatures reach 300°C. Up to 600°C enamel will turn dark brown and then black as carbonisation is reached. Dentin will turn black as it is carbonised, with no further colour changes seen after this stage. At temperatures of >600°C, enamel will begin to turn a light grey colour, indicating calcination, after which no further colour changes will be seen (44, 67, 69-71).

TAPHONOMY IN AQUATIC ENVIRONMENTS

Aquatic environments include both freshwater (fluvial, lacustrine, groundwater etc.) and saltwater (marine) systems, where remains can be deposited as a result of homicides, suicides, accidents or burials at sea (72, 73). Whilst aquatic environments may become a secondary depositional location for remains, only those deposited into aquatic environment as a primary location will be considered in this review, due to a lack of research surrounding

the taphonomic histories of remains deposited secondarily. Artificial environments, such as suitcases, bags, and situations where bodies have been weighted down or mutilated in any way, will be excluded from this review due to the complexity of the described scenarios, and what little current knowledge is known surrounding their decomposition rates and taphonomic histories.

Freshwater. Freshwater aquatic environments involve a range of sources, including rivers, wetlands, lakes, and groundwater systems. These environments are typically free-flowing and fast moving, as opposed to the tidal movement of saltwater systems, and can thus transport remains long distances in short periods of time, resulting in varying levels of sediment abrasion. Freshwater systems also tend to have more variable pH levels compared to that of saltwater, which can result in acidic corrosion or excellent preservation of remains.

Fluvial systems can transport remains across great distances, even depositing them into the ocean (76). Transport is generally episodic due to water level fluctuations, and the remains can potentially become embedded in the riverbed or trapped with other structures, such as logs, rocks, or debris (72). Depending on the mechanisms and force of transport, different levels of sediment abrasion may be seen on bones. Rounding and smoothing of protruding edges is common, along with small scratches, pitting, chipping, and denting of the cortical surface. Long grooves and fractures are rarely seen, though can be produced if the force of movement is strong enough. Natural openings within the bone, such as vascular canals and foramina, will gradually become enlarged over time, and in juvenile remains, epiphyses may detach if not fused. As the cortical layer thins, the underlying trabecular bone may also be exposed towards the epiphyseal ends. Rivers with large, coarse sediments will impart

increased levels of damage upon bone, whilst those with fine sandy or silty sediments will produce more subtle damage. These sediments can also become embedded within bone, into any natural openings, grooves, pits, or exposed trabecular bones from the processes of sediment abrasion. This sediment impaction can provide information to investigators as to where the remains were deposited at one point in their taphonomic history (72).

Not much research has been conducted regarding the effects of varying pH levels of water on bone structure, and whether pH would play a significant role in the degradation of bone in a fluvial environment. An experimental study assessed the impacts that pH had over a year-long period. The best levels of preservation were seen at pH 7 and 10, whilst the poorest preservation was seen at pH 1 and 14, with the bone specimens completely dissolved after just 3 weeks at pH 1. Acid etching due to low pH appears as pitting and roughening on the surface, often accompanied by discolouration. These results indicate that different considerations would have to be made regarding the taphonomic histories of aquatic specimens, and subsequent post-mortem submersion interval (PMSI) and PMI ranges, based on the pH of the depositional environment (78).

Saltwater. Saltwater aquatic environments are less varied than those of freshwater, and typically include the ocean, saltwater lakes, and marshes. The tidal movements of these systems can contribute to taphonomic damage like sediment abrasion and bioerosion, as remains are battered against the ocean floor and other debris, and various marine taxa adhering to bone to use as a location for growth and feeding. Further, the interaction between the salt and the sun's UV rays can lead to a bleaching, a more uniform display that is seen in subaerial remains, whilst various isotopes and REEs present in the water will gradually alter the chemical signature of the biomineralised tissues, resulting in a structure

reflecting that of the surrounding depositional environment. The pH of saltwater, whilst less variable than those seen in freshwater environments, lingering around a slightly alkaline range of 8.1-8.2, can potentially preserve bone indefinitely; however, collagen will still decay and disappear over time as it is exchanged with minerals in the environment, although much slower than in subaerial, burial or burning environments (78).

UV exposure. Bones deposited into the marine environment will experience near constant exposure to UV rays from the sun, leading to increased levels of bleaching over time; the result of a chemical reaction between the UV light from the sun, and the salt water, alongside the gradual loss of organic content (73, 76). Marine bleaching can be differentiated from subaerial bleaching through analysis of other taphonomic effects present on the bone. Subaerial bleaching will often be accompanied by cracking, flaking, and exfoliation of the cortical surface, with only a portion affected by UV exposure. In comparison, marine bleaching will be uniform (73).

Encrustation and bioerosion. Various marine taxa will adhere to hard substrates, such as bone, for support or to feed upon other adhering taxa or on the bone itself, resulting in characteristic damage that can oftentimes be traced back to the individual taxa that caused it (73, 74, 76). The presence of adherent marine taxa on bone supports the theory that the bones were deposited in a marine environment at some point during their taphonomic history. Some marine taxa can also be used in the estimation of PMSI and PMI ranges, based on their degree of growth (73). Common species known to adhere to bone include algae, coral, Bryozoa, molluscs and barnacles.

Algae and Bryozoa are easily identifiable, especially bryozoans, which may leave behind a hard organic or mineral 'shell' on bone once the organism has died. Coral, molluscs, and barnacles can be identified through their hard calcium carbonate shells, feeding and homing scars, and calcified plates, respectively. Molluscs feed on bone substrates, using a chitinous feeding appendage lined with small teeth (radula), which scrapes away at the bone surface leaving behind a series of parallel striations approximately 20-100µm in depth. Sea urchins, or echinoids, use their five-toothed jaw apparatus to remove bone, resulting in a star-shaped pattern of grooves along the bone surface (73).

Common microborers include *Osedax* worms, osteophagous crabs such as the tanner crabs, fungi, bacteria, and algae (73, 74, 76). *Osedax* and *Polychaete* worms utilise heterotrophic bacterial endosymbionts or proteolytic enzymes, respectively, to break down the complex organic components of bone for feeding (81). This activity leads to the formation of tunnels through bone, though these worms will also produce tunnels as a form of protective housing (76). Fungi, bacteria and algae commonly produce Wedl-type tunnelling, and also act as a food source to larger species, which encourages grazing upon bone (73).

Sediment abrasion. The constant and highly energetic movement of coastal waters can lead to the impaction of bone against multiple substrates, resulting in characteristic damage (73, 76). As bone comes into contact with the ocean floor sediment, or sharp, rocky areas, its surface will gradually accumulate damage, such as rounding, cortical thinning which may expose underlying trabecular bone, loss of unfused elements like epiphyses, and surface damage like scratches, pitting or indentations (74, 76). Any natural openings within a bone, like vascular canals and foramina, will gradually expand in a process called "windowing" (76). The rate of abrasion will generally depend on the composition of the sediment surrounding

the bone. Coarser sediments have been demonstrated to produce greater abrasive damage over shorter time periods than finer sediments, though the degree of damage caused by these sediments is also dependent on the duration of exposure, abrasive force, bone hardness and elasticity as well as the sphericity of the sediment grains (74).

Isotopic and REE concentrations. The isotopic composition of bones, altered gradually by the surrounding saltwater environment, can be used to reconstruct factors such as perimortem diet and migration behaviours, as well as the specific nature of the post-mortem depositional environment (82, 83). Certain isotopes obtained from BA within bones can provide information regarding the climatic and environmental conditions of temperature and humidity that affected the bones during deposition (82, 83). These isotopes are incorporated into the BA from the surrounding saltwater through recrystallisation, as the bone undergoes natural diagenesis (83). This diagenetic alteration takes many years to occur, to a point where levels are altered enough to show a clear difference between the natural bone isotopic concentrations, and concentrations reflecting the surrounding depositional environment (83).

Rare earth elements (REEs) can similarly be used in the identification of a bone's taphonomic history, as REE concentrations are reflective of a particular environment and geographical location, creating a unique diagenetic 'signal' (38). Bones deposited in a specific environment for long periods of time will eventually reflect a REE concentration very similar to that of the surrounding pore water (83). Variations in chemical abundances are less pronounced in aquatic environments, and differ even between fluvial and marine settings, which should be taken into account when assessing REE ratios as a form of taphonomic analysis (73, 84).

TAPHONOMY IN FROZEN ENVIRONMENTS

Frozen environments define those with an ambient temperature below 0°C (32°F), often with the presence of ice or snow, for part or all the calendar year. Human remains found in these environments are often the result of accidental deaths, such as when hiking or because of a plane crash, due to the remoteness and harsh nature of frozen landscapes (85).

Freeze-thaw cycles. The formation of ice within the small bone spaces not only removes moisture from the bone matrix but can also lead to structural weakening and the formation of microcracks. When this ice melts, and refreezes with subsequent freeze-thaw cycles (e.g., due to seasonal fluctuations), it leaves more space to be filled within the bone, leading to a further expansion and structural damage (90). In Tersigni (2007) (91), microfractures were identified within the Haversian canal systems of frozen bone samples, absent from all controls. Turpin (2017) (86) also noted these microfractures in samples of human bone frozen for 21 days. Two distinct types of microfracturing were observed, including irregularly shaped transverse cracks along the interior cortical edge, and linear osteonal cracks originating in the Haversian canal system of a single osteon. Of interest was the lack of microfracturing seen in human bones that had been embalmed prior to the freeze-thaw experiment (86). Whilst research surrounding the taphonomy of teeth in frozen environments is scant, one study (87) found mammoth teeth to be intact and without any post-mortem damage after thousands of years buried beneath the permafrost.

Freeze-drying (mummification). When remains undergo rapid freezing and drying, they are essentially mummified, because the process of decomposition ceases so promptly after death (86). Rapid freezing removes moisture from the intracellular and extracellular spaces within

the body, as it is transformed into ice crystals. This results in the dehydration of tissues and subsequent mummification of the remains (88). With little interference from the standard taphonomic agents found in many other depositional environments, such as increased temperatures, weathering, and insect or animal activities, remains can essentially persist in a mummified state until recovery (88).

4. DISCUSSION

Forensic taphonomy, a sub-discipline of forensic anthropology, studies the post-mortem modifications which affect human and non-human remains, and are produced by a variety of taphonomic agents of physical, chemical and biological nature (10). This study reviewed the current literature to compile to-date information on the taphonomic agents present across the five most common depositional environments, and the effects generated on biomineralized tissues, to assist the forensic community in reconstructing the post-mortem histories of skeletal remains in forensic investigations.

Whilst some environments share similar taphonomic agents, other taphonomic effects are unique to specific depositional environments. Burial environments, in which deceased individuals are deposited into a grave, either a formal coffin burial or the placement into a clandestine pit, demonstrated a few key taphonomic effects (14). Buried biomineralized tissues may exhibit evidence of bioerosion (e.g., by bacteria), degradation due to soil characteristics (e.g., pH), mineral staining from soil particulates or coffin hardware, and coffin wear. Subaerial environments, with remains placed at the ground surface and exposed to the natural elements (30, 31), are associated with characteristic effects, such as weathering from fluctuating climatic conditions, delamination, cracking, bleaching of exposed surfaces due to increased UV exposure, and bioerosion (e.g., by plants). Burnt remains present different

taphonomic effects (41) depending on the range of temperatures and the duration of the fire, as well as the condition of the body (i.e., fresh, decomposing, skeletonised). Bone and teeth may exhibit intense colour changes from an initial pale yellow, through to brown and black (carbonised) and white/grey-blue (calcined). Accompanying colour changes are heat-related fractures, and a reduction in the organic fraction of tissues. Histological and crystallographic analyses may also show changes in the micro- and nanostructure of bone and teeth. Frozen environments, defined as those with an ambient temperature below 0°C (32°F) and with the presence of ice or snow for most of the calendar year (85), feature limited taphonomic research, however common factors affecting bones and teeth include freeze-thaw cycles, mummification due to the decreased temperature reducing or stopping decomposition, and damages linked to patterns of movement and soil composition typical of glaciers. In aquatic environments, biomineralized tissues may undergo several characteristic alterations depending on the type of water they are deposited in, that is freshwater or saltwater. Common effects observed in freshwater include sediment abrasion, degradation by water lower pH levels, and discolouration. Similarly, in a marine environment, bones and teeth may undergo complete bleaching (as opposed to the partial bleaching seen in subaerial environments), encrustation and bioerosion (e.g., from adhering and colonizing taxa), altered isotope and REE concentrations, and sediment abrasion (72, 73).

When biomineralized tissues are recovered from one of the listed environments, the first step is to determine whether they are of human or non-human origin. This is usually achieved by forensic pathologists and anthropologists, who are trained in recognising human bones and teeth. Human bones, particularly those of the hand, can commonly be mistaken for animal bones, such as a bear paw (8). This is especially evident when the remains have been scattered by scavenging activities. Once the human species is confirmed, the next steps aim towards

personal identification and the assessment of the circumstances surrounding death, including estimating the PMI. Forensic investigators employed by the local law enforcement will conduct the investigation surrounding the circumstances of death, how the recovery location was reached, and the PMI. The forensic anthropologist will produce a biological profile, with the determination of sex, age, living stature and pathological traits, which may lead to either a presumptive or a positive identification. This interpretation is supported by research gathered from taphonomic research facilities (commonly known as “body farms”), which are established with the aim of placing donated human bodies in various natural and artificial depositional environments, to study the rate and process of decomposition and taphonomic alteration in response to such variations. Currently, the variety of these facilities is limited, with only one cold climate farm established in Canada, one subtropical farm in Australia, and several others across the USA. Such research facilities become inadequate when considering the array of other environments where research is lacking, such as aquatic, arid, frozen, and tropical environments, especially from an Australian perspective, considering that the vastity of the country and the different environments present.

Whilst forensic taphonomy can similarly provide an insight into the circumstances surrounding death and an estimation of PMI, it can also supply information about the post-mortem history of the remains. By understanding the environment-specific agents that damage bones and teeth, a more detailed analysis of the depositional environment can be produced. The location or the timeline of the deposition of the remains may not match other evidence collected in the investigation, therefore a thorough analysis of the taphonomic damage to bones and teeth may indicate and clarify the differences (92). This information may then assist investigators by narrowing down the location and the time of deposition. Forensic taphonomy can also provide an insight into which analytical techniques can be

applied to remains, for example genetics. In some circumstances, the age of the remains or severe damage (such as through extreme subaerial weathering or thermal alteration), will not allow the extraction of DNA, or accurate measurement of the bones (57, 67). This information can save time and costs associated with running such analyses and can lead to different analytical techniques better suited to the specific samples.

Forensic taphonomy is not without limitations. Being a relatively new sub-discipline of forensic anthropology, it features several areas where research is quite scarce, or just emerging. Taphonomic investigations are limited for teeth in comparison to those available for bones. The damage to bones and teeth deposited in burial, coffin, aquatic, and frozen environments is also understudied, alongside specific analytical techniques, such as DNA analysis, on bone and teeth that have been deposited into the environment for long periods, or that have undergone severe degradation, including thermal alteration and subaerial weathering. As more research is conducted in forensic taphonomy, the benefit of incorporating this subdiscipline into the workflow of forensic investigations is growing in relevance.

5. CONCLUSION

This study compiled and analysed the to-date information on the taphonomic processes that affect biomineralized tissues, bones and teeth, across the five most common depositional environments: burial, subaerial exposure, aquatic environments, burnt and frozen remains. The unique taphonomic effects observed in each depositional environment can assist in determining a more accurate description of the post-mortem history of human or non-human skeletal remains. As further research is conducted in this field, the benefit of incorporating forensic taphonomy knowledge into forensic investigations becomes increasingly apparent.

The techniques of a forensic taphonomic investigation are not widely taught, even to forensic anthropologists. By enacting a multi-disciplinary approach to investigations through the inclusion of forensic taphonomy, including the provision of training to current forensic anthropologists and investigators, a more thorough investigation can be undertaken, which may allow for better outcomes in the reconstruction of post-mortem histories, identification of remains and overall resolution of cases.

6. REFERENCES

1. Hu J, Liu X, Ma PX. Biomineralisation and Bone Regeneration. Second ed. Anthony Atala RL, James A. Thomson, Robert Nerem, editor: Academic Press; 2011.
2. Crichton RR. Biomineralisation. Second ed. Crichton RR, editor: Elsevier; 2012.
3. Senior KR. Bone and Muscle: Structure, Force, and Motion: The Rosen Publishing Group, Inc; 2010.
4. Houck MM, Siegel JA. Fundamentals of forensic science: Academic Press; 2009.
5. Pawlina W, Ross MH. Histology: a text and atlas: with correlated cell and molecular biology: Lippincott Williams & Wilkins; 2018.
6. Kono T, Sakae T, Nakada H, Kaneda T, Okada H. Confusion between Carbonate Apatite and Biological Apatite (Carbonated Hydroxyapatite) in Bone and Teeth. Minerals. 2022;12(2):170.
7. Ubelaker DH. The forensic evaluation of burned skeletal remains: A synthesis. Forensic science international. 2009;183(1-3):1-5.
8. Byers SN. Introduction to forensic anthropology: Taylor & Francis; 2016.
9. Schotsmans EM, Márquez-Grant N, Forbes SL. Taphonomy of human remains: forensic analysis of the dead and the depositional environment: John Wiley & Sons; 2017.
10. Dupras TL. Forensic recovery of human remains: archaeological approaches: CRC Press; 2005.
11. POKINES JT. Introduction: Collection of macroscopic osseous taphonomic data and the recognition of taphonomic suites of characteristics. Manual of forensic taphonomy: CRC Press; 2013. p. 16-33.
12. Pearson MP, Pearson MP. The archaeology of death and burial: Sutton Phoenix Mill, UK; 1999.
13. Pokines JT, Zinni DP, Crowley K. Taphonomic patterning of cemetery remains received at the Office of the Chief Medical Examiner, Boston, Massachusetts. Journal of forensic sciences. 2016;61:S71-S81.
14. Pokines JT, Baker JE. Effects of burial environment on osseous remains. Manual of forensic taphonomy. 2013:73-114.
15. Buekenhout I, Vieira DN, Ferreira MT. Reliability of weathering in the estimation of the post-mortem interval of human remains buried in coffins. Australian Journal of Forensic Sciences. 2018;50(4):414-27.
16. Egeland CP, Pickering TR. Cruel traces: Bone surface modifications and their relevance to forensic science. Wiley Interdisciplinary Reviews: Forensic Science. 2021;3(3):e1400.
17. Nicholson RA. Bone degradation, burial medium and species representation: debunking the myths, an experiment-based approach. Journal of Archaeological Science. 1996;23(4):513-33.
18. Child AM. Microbial taphonomy of archaeological bone. Studies in conservation. 1995;40(1):19-30.
19. Fisher JW. Bone surface modifications in zooarchaeology. Journal of Archaeological method and theory. 1995;2(1):7-68.
20. Alexandersen V, G. Norén J, Hoyer I, Dietz W, Johansson G. Aspects of teeth from archaeologic sites in Sweden and Denmark. Acta Odontologica Scandinavica. 1998;56(1):14-9.
21. Kendall C, Eriksen AMH, Kontopoulos I, Collins MJ, Turner-Walker G. Diagenesis of archaeological bone and tooth. Palaeogeography, Palaeoclimatology, Palaeoecology. 2018;491:21-37.
22. Pokines JT, Faillace K, Berger J, Pirtle D, Sharpe M, Curtis A, et al. The effects of repeated wet-dry cycles as a component of bone weathering. Journal of Archaeological Science: Reports. 2018;17:433-41.
23. Behrensmeyer AK. Taphonomic and ecologic information from bone weathering. Paleobiology. 1978;4(2):150-62.

24. Madgwick R, Mulville J. Investigating variation in the prevalence of weathering in faunal assemblages in the UK: a multivariate statistical approach. International Journal of Osteoarchaeology. 2012;22(5):509-22.
25. Vietti LA. Quantifying bone weathering stages using the average roughness parameter Ra measured from 3D data. Surface Topography: Metrology and Properties. 2016;4(3):034006.
26. JUNOD CA, POKINES JT. Subaerial weathering. Manual of forensic taphonomy: CRC Press; 2013. p. 302-29.
27. Stokes S, Márquez-Grant N, Greenwood C. Establishing a minimum PMI for bone sun bleaching in a UK environment with a controlled desert-simulated comparison. International Journal of Legal Medicine. 2020;134(6):2297-306.
28. Trueman CN, Behrensmeyer AK, Tuross N, Weiner S. Mineralogical and compositional changes in bones exposed on soil surfaces in Amboseli National Park, Kenya: diagenetic mechanisms and the role of sediment pore fluids. Journal of Archaeological Science. 2004;31(6):721-39.
29. Guareschi EE, Nicholls PK, Evans NJ, Barham M, McDonald BJ, Magni PA, et al. Bone diagenesis in the marine environment-I: characterization and distribution of trace elements in terrestrial mammalian bones recovered from historic shipwrecks. International Journal of Osteoarchaeology.
30. Fernández-Jalvo Y, Andrews P, Pesquero D, Smith C, Marín-Monfort D, Sánchez B, et al. Early bone diagenesis in temperate environments: Part I: Surface features and histology. Palaeogeography, Palaeoclimatology, Palaeoecology. 2010;288(1-4):62-81.
31. Symes SA, L'abbé EN, Pokines JT, Yuzwa T, Messer D, Stromquist A, et al. Thermal alteration to bone. Manual of forensic taphonomy: CRC Press; 2013. p. 382-417.
32. Symes SA, Rainwater CW, Chapman EN, Gipson DR, Piper AL. Patterned thermal destruction of human remains in a forensic setting. The analysis of burned human remains: Elsevier; 2008. p. 15-vi.
33. Shipman P, Foster G, Schoeninger M. Burnt bones and teeth: an experimental study of color, morphology, crystal structure and shrinkage. Journal of archaeological science. 1984;11(4):307-25.
34. Ellingham ST, Thompson TJ, Islam M, Taylor G. Estimating temperature exposure of burnt bone—A methodological review. Science & Justice. 2015;55(3):181-8.
35. Shahack-Gross R, Bar-Yosef O, Weiner S. Black-coloured bones in Hayonim Cave, Israel: differentiating between burning and oxide staining. Journal of archaeological Science. 1997;24(5):439-46.
36. Dunlop JM. Traffic light discoloration in cremated bones. Medicine, Science and the Law. 1978;18(3):163-73.
37. Squires KE, Thompson TJ, Islam M, Chamberlain A. The application of histomorphometry and Fourier Transform Infrared Spectroscopy to the analysis of early Anglo-Saxon burned bone. Journal of Archaeological Science. 2011;38(9):2399-409.
38. Castillo RF, Ubelaker DH, Acosta JAL, de la Fuente GAC. Effects of temperature on bone tissue. Histological study of the changes in the bone matrix. Forensic science international. 2013;226(1-3):33-7.
39. Piga G, Thompson TJ, Malgosa A, Enzo S. The potential of X-ray diffraction in the analysis of burned remains from forensic contexts. Journal of Forensic Sciences. 2009;54(3):534-9.
40. Figueiredo M, Fernando A, Martins G, Freitas J, Judas F, Figueiredo H. Effect of the calcination temperature on the composition and microstructure of hydroxyapatite derived from human and animal bone. Ceramics international. 2010;36(8):2383-93.
41. Snoeck C, Lee-Thorp J, Schulting R. From bone to ash: Compositional and structural changes in burned modern and archaeological bone. Palaeogeography, palaeoclimatology, palaeoecology. 2014;416:55-68.
42. Mckinnon M, Henneberg M, Simpson E, Higgins D. A comparison of crystal structure in fresh, burned and archaic bone–Implications for forensic sampling. Forensic Science International. 2020;313:110328.

43. Bonucci E, Graziani G. Comparative thermogravimetric, x-ray diffraction and electron microscope investigations of burnt bones from recent, ancient and prehistoric age. Atti della Accademia Nazionale dei Lincei Classe di Scienze Fisiche, Matematiche e Naturali Rendiconti. 1975;59:517-32.
44. Harbeck M, Schleuder R, Schneider J, Wiechmann I, Schmahl WW, Grupe G. Research potential and limitations of trace analyses of cremated remains. Forensic science international. 2011;204(1-3):191-200.
45. Neson R. A microscopic comparison of fresh and burned bone. Journal of Forensic Science. 1992;37(4):1055-60.
46. Correia PM. Fire modification of bone: a review of the literature. Forensic taphonomy: The postmortem fate of human remains. 1997:275-93.
47. Herrmann B. Über die Abhängigkeit der Schrumpfung vom Mineralgehalt bei experimentell verbrannten Knochen. Anthropologischer Anzeiger. 1977:7-12.
48. Herrmann B. On histological investigations of cremated human remains. Journal of Human Evolution. 1977;6(2):101-3.
49. Herrmann B. Neuere Ergebnisse zur Beurteilung menschlicher Brandknochen. Zeitschrift für Rechtsmedizin. 1976;77(3):191-200.
50. Rubio L, Sioli JM, Gaitán MJ, Martin-de-Las-Heras S. Dental color measurement to predict DNA concentration in incinerated teeth for human identification. PloS one. 2018;13(4):e0196305.
51. Beach JJ, Passalacqua NV, Chapman EN. Heat-related changes in tooth color: temperature versus duration of exposure. The analysis of burned human remains: Elsevier; 2008. p. 137-xi.
52. Wilson DF, Massey W. Scanning electron microscopy of incinerated teeth. The American Journal of Forensic Medicine and Pathology. 1987;8(1):32-8.
53. Garriga JA, Ubelaker DH, Zapico SC. Evaluation of macroscopic changes and the efficiency of DNA profiling from burnt teeth. Science & Justice. 2016;56(6):437-42.
54. Bromage TG, Goldman HM, McFarlin SC, Warshaw J, Boyde A, Riggs CM. Circularly polarized light standards for investigations of collagen fiber orientation in bone. The Anatomical Record Part B: The New Anatomist: An Official Publication of the American Association of Anatomists. 2003;274(1):157-68.
55. Collins MJ, Nielsen–Marsh CM, Hiller J, Smith C, Roberts J, Prigodich R, et al. The survival of organic matter in bone: a review. Archaeometry. 2002;44(3):383-94.
56. Jellinghaus K, Urban PK, Hachmann C, Bohnert M, Hotz G, Rosendahl W, et al. Collagen degradation as a possibility to determine the post-mortem interval (PMI) of human bones in a forensic context–A survey. Legal Medicine. 2019;36:96-102.
57. Higgins D, Rohrlach AB, Kaidonis J, Townsend G, Austin JJ. Differential nuclear and mitochondrial DNA preservation in post-mortem teeth with implications for forensic and ancient DNA studies. PloS one. 2015;10(5):e0126935.
58. Schmidt CW. Burned human teeth. The analysis of burned human remains: Elsevier; 2015. p. 61-81.
59. Evans T. Fluvial taphonomy. Manual of forensic taphonomy: CRC Press; 2013. p. 130-57.
60. Higgs ND, Pokines JT. Marine environmental alterations to bone: CRC Press Boca Raton, FL; 2014.
61. Pokines JT, Higgs N. Macroscopic taphonomic alterations to human bone recovered from marine environments. J Forensic Identif. 2015;65(6):953-84.
62. Christensen AM, Myers SW. Macroscopic observations of the effects of varying fresh water pH on bone. Journal of forensic sciences. 2011;56(2):475-9.
63. Pokines JT, Menschel M, Mills S, Janowiak E, Satish R, Kincer C. Experimental Formation of Marine Abrasion on Bone and the Forensic Postmortem Submergence Interval. Forensic Anthropology. 2020;3(4):175.

64. Belaústegui Z, de Gibert JM, Domènech R, Muñiz F, Martinell J. Clavate borings in a Miocene cetacean skeleton from Tarragona (NE Spain) and the fossil record of marine bone bioerosion. Palaeogeography, Palaeoclimatology, Palaeoecology. 2012;323:68-74.
65. Zazzo A, Lécuyer C, Mariotti A. Experimentally-controlled carbon and oxygen isotope exchange between bioapatites and water under inorganic and microbially-mediated conditions. Geochimica et Cosmochimica Acta. 2004;68(1):1-12.
66. Tütken T, Vennemann T, Pfretzschner H-U. Early diagenesis of bone and tooth apatite in fluvial and marine settings: constraints from combined oxygen isotope, nitrogen and REE analysis. Palaeogeography, Palaeoclimatology, Palaeoecology. 2008;266(3-4):254-68.
67. Trueman C, Benton MJ, Palmer M. Geochemical taphonomy of shallow marine vertebrate assemblages. Palaeogeography, Palaeoclimatology, Palaeoecology. 2003;197(3-4):151-69.
68. Pilloud MA, Megyesi MS, Truffer M, Congram D. The taphonomy of human remains in a glacial environment. Forensic Science International. 2016;261:161. e1-. e8.
69. Hale AR, Ross AH. The impact of freezing on bone mineral density: implications for forensic research. Journal of forensic sciences. 2017;62(2):399-404.
70. Tersigni MA. Frozen human bone: a microscopic investigation. Journal of forensic sciences. 2007;52(1):16-20.
71. Turpin C. The Micro-Taphonomy of Cold: Differential Microcracking in Response to Experimental Cold-Stresses. Journal of forensic sciences. 2017;62(5):1134-9.
72. Maschenko E, Potapova O, Heintzman P, Kapp J, Shapiro B, Protopopov A, et al. Morphology, Individual Age, DNA and Sex of the Yuka Mammoth (Mammuthus primigenius) from Northern Yakutia, Russia. Paleontological Journal. 2021;55(11):1230-59.
73. Roberts LG, Dabbs GR. A Taphonomic Study Exploring the Differences in Decomposition Rate and Manner between Frozen and Never Frozen Domestic Pigs (S us scrofa). Journal of forensic sciences. 2015;60(3):588-94.
74. Pokines JT, L'Abbe EN, Symes SA. Manual of forensic taphonomy: CRC Press; 2021.