Masters in Forensic Science Dissertation Thesis

A REVIEW OF THE USE OF *SUS SCROFA* AS AN ANALOGUE TO HUMAN DECOMPOSITION STUDIES

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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 o 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: Tracie Su-Lin Narayan Date: 6 Dec 2021

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Part One

Literature Review

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1. Abstract

Taphonomy comes from the Greek word taphos (τάφος) meaning grave but has more commonly been accepted as the study of an organism from the time of its death to the point of its discovery. A multifaceted area, it incorporates decomposition, burial, transportations, and the chemical, physical, and biological factors that go alongside. In many experimental studies, animals are used as analogues when human remains are either unavailable or unlicensed. At research facilities across the globe, such as the Wrexham Glyndŵr University's Forensic Science and Crime Scene Research Area, other easily accessible mammals are used in the place of human cadavers. Most research is commonly conducted on pigs, rabbits, mice, or rats, either dealing with the carcass as a whole or select anatomic sections, for example, trotters. The choice of these is often dependent on the cost, availability, and scale of the experiment. While these studies present intriguing patterns, there is still a need for research conducted within human samples, using larger sample sizes, and in differing environments. Additionally, longitudinal studies are warranted in dry areas where desiccation occurs, and bodies take much longer than 5 months to skeletonize. With further research, it may be possible in the future to use animal models as accurate analogues for human cadavers to measure specific measurements. For example, pigs have shown similar insect successions to humans, and insects of forensic interest are also common to dog. It may become possible to tailor decomposition studies using animal models to more precisely mimic specific artifacts of human decomposition.

2. Forensic Taphonomy

Taphonomy comes from the Greek word taphos (τάφος) meaning grave but has more commonly been accepted as the study of an organism from the time of its death to the point of its discovery. A multifaceted area, it incorporates decomposition, burial, transportations, and the chemical, physical, and biological factors that go alongside (1). Biotaphonomy is focused around aspects such as decomposition, preservation, and modification. Several variables constantly affect the biotaphonomic action occurring. There are external factors that are both abiotic, such as climate, and biotic, such as animal activity, as well as individual factors such as height and weight. With prolonged deterioration, there is leeching of material into the deposition environment and this is where we enter the remit of geotaphonomy. The cadaver itself forms a microenvironment caused by its decay, integration or any scattering, complex trophic phases that can initiate variations to the chemistry and ecology of the surrounding environment. Differences that characterize this environment, such as weather, microbiology, and body coverings, all form a significant impact on the taphonomic processes, some of which can act as a catalyst to the overall process of decomposition (1, 2).

2.1 Stages of Decomposition

2.1.1 Autolysis

Decomposition begins initially within the abdomen because of the high diversity and abundance of hydrolytic enzymes from the liver, stomach, and pancreas, which begin to break down cells and tissues. The decreased concentrations of oxygen in the body, along with the increase of carbon dioxide and other waste products, cause a shift in pH within cells. This leads

to a subsequent decreased integrity of the cellular membranes, resulting in enzymes being released and denaturing the remaining membrane molecules *(3). Many of these microscopic and chemical changes are not observed easily at this stage without histological testing. This confirms the degradation of the cell structure and provides evidence of cell necrosis. Often more macroscopic characteristics such as clouding in the cornea, cooling of the body or other observable characteristics can be noted externally (4). Overall, many features of both putrefaction and autolysis occur simultaneously and therefore it can be difficult to attribute them to the separate processes. Technically, however, autolysis is enzyme driven with putrefaction resulting in the activity of bacteria. Notwithstanding these differences, both features contribute to the overall taphonomic changes witnessed in the cadaver.*

2.1.2 Algor mortis

Algor mortis, the cooling of the body post-mortem, is the result of a cessation in thermoregulation. As body temperature is controlled by the hypothalamus, this homeostatic feature can no longer be maintained after death. Thus, the body temperature will begin to change toward the ambient temperature of the room or surroundings in which the remains are found. This occurs through radiation, convection, conduction, and, if the subject is wet, evaporation. Most commonly, the body temperature decreases, although in more extreme climates an increase in cadaver temperature may result. Consequently, temperature change can play a particularly important role in establishing the PMI, which in turn helps to reconstruct the chronology and circumstances surrounding death (5). Although a generally accepted principle is that body temperature decreases by an average of 1°C per hour, research findings

suggest that this process can be delayed by up to 3 h in the initial post-mortem period (6). Newton's Law of Cooling states that the larger the temperature difference between an object and its surrounding, the quicker it will cool. However, it has been well accepted that this pattern should not be adapted for cadavers with Rainy (1868) (7) first recording that cadaveric cooling did not occur in this fashion. This was later attributed to the continuation of metabolic processes and the complexities of interior and surface cooling, resulting in nonuniform changes across the body being recorded (8).

It has also been noted that such standardized curves may not consider other factors such as natural differences in body temperature at the time of death, wind speed, and surface area-to-volume ratio. For example, thinner individuals tend to cool much more quickly. Individual variations such as these have led to suggestions that this PMI estimation approach holds little value when applied to human remains in real criminal investigations (9).

One of the most renowned tools for PMI estimations from body temperature is the nomogram method. The approach adopts separate graphs that are provided for ambient temperatures above and below 23 °C and considerations such as clothing and water can be taken into account with the lag phase also corrected for (10). Concerns over the use of algor mortis as a feasible method for PMI estimation remain particularly for death scenes where significant abdominal trauma or potential sexual assault has occurred. This is because the methods for measuring post-mortem body temperature rely on the penetration of internal organs or rectal thermometer readings, which can be either impossible or unsuitable depending on the nature of the crime. Additionally, depending on the amount of microbial and entomological activity, accurate body temperatures cannot always be established as maggot masses and bacteria

activity may generate heat. Therefore, the use of body temperature as a method for determining PMI should be restricted to fresh remains.

2.1.3 Livor mortis

Another characteristic of the fresh stage of decomposition is the appearance and fixation of livor mortis, also known as hypostasis or lividity. As for algor mortis, lividity is a result of normal bodily functions ceasing, but with a focus on the lack of circulation and oxygen depletion. Normally observed within 2 h of death, but sometimes as quickly as the first 20 min, lividity is a characteristic discoloration to the skin caused by the pooling of blood (11). Without cardiac contractions to keep it moving, blood, whose oxygen has dissociated from its hemoglobin, collects in the lower parts of the body causing the skin to appear red or dark purple. Initially, blood remains fluid due to the presence of plasmin, a fibrinolytic enzyme that prevents coagulation by reducing fibrinogen levels. Thus blood can be moved easily and forced out of different parts of the body due to pressure caused by contact with surfaces, such as a hard floor, or around tight-fitting ligatures. Sometimes even aspects of clothing such as elasticated waistbands can cause enough pressure to leave white voids within the tissue staining. This unique patterning becomes fixed on the skin due to hemolysis within 12 h and can prove a very useful tool in determining the location, position, and any postmortem movement of the body. Nevertheless, the timing and presence of livor mortis is highly unreliable for accurate use in PMI determination. Although studies have been conducted on their potential use, spectrophotometry and colorimetry are still extremely limited in their practical capacity (12).

A secondary effect of hypostasis is a characteristic known as pallor. As blood begins to pool, it drains away from capillaries close to the skin's surface on the superior aspects of the body. Much like the paling complexion as a heat preservation mechanism in living individuals, a colour loss can be observed in the skin resulting in a more greyed appearance, hence the term "deathly pale" (13).

2.1.4 Rigor mortis

Rigor mortis is possibly one of the most well-known of the taphonomic changes and is the process that causes the muscles in the body to stiffen resulting in rigidity due to a range of chemical changes in the muscle structure. Muscle fibres, which in life move because of sliding filament theory, rely on the conversion of ATP to ADP. After death, when respiration ceases, the intracellular pH decreases due to the production of lactic and pyruvic acid. The anaerobic glycolysis of glycogen in the muscles causes glycogen depletion and thus reduced ATP concentrations. Also, calcium leaks into the sarcomere, where the protein filaments of actin and myosin are present in an alternating arrangement, where calcium then binds allowing for a cross-linkage to occur between the filaments. This causes a pulling motion along the length of the muscle causing it to become shorter and more rigid. In a living individual, ATP would be used to dissociate the cross-linking in the fibres and as a result the rigidity associated with the change would be reversed, whereas it becomes fixed post-mortem (14).

First noticed in the small muscle groups such as in the hands and face, rigor can take hold within 3 to 4 h and normally extends across the large muscle groups also within the first 12 h after death, causing the entire body to become stiff. Variations in this time period are observed

particularly in individuals who may have decreased levels of ATP at the point of death. This could be caused by exercise or strenuous activity in the perimortem period around the time of death (15).

Rigor mortis can also be helpful in the reconstruction of the postmortem period by preserving the positioning of the body and in some circumstances showing if any attempt has been made to move the body. However, this type of interpretation is very much time dependent and relies on rigor still being present at the time the body is discovered. The point at which this rigidity reverses and the stiffness passes is extremely variable because of significant differences in the volume of muscle in the remains. However, the body generally returns to a fully flaccid state after 36 h from the time of death, although this can increase by up to 10 days in refrigerated remains (16).

2.1.5 Bloat and Putrefaction

As the primary autolytic stage ends, the body has become a nutrient-rich, acidic, and anaerobic environment. Additionally, the normal defense mechanisms, such as the immunological response against foreign agents, have ceased. This allows exogenous and endogenous microorganisms to thrive inside and on the cadaver. Many of these are natural populations from inside the body but others may migrate in from the surrounding environment. These increased microbial communities start to break down body tissues, producing gases and other metabolic by-products that are responsible for the bloating and unpleasant odor that are characteristic of this stage. Overall, the complex biological molecules within the soft tissue are

broken down by the microbes into liquids and gases resulting in an overall loss of integrity and structure (17).

Much of this activity starts in the digestive system, which contains a range of microbial species such as Staphylococcus epidermidis, Clostridium perfringens, and Escherichia coli. One of the significant observable changes of this stage is the increased production of gases such as hydrogen sulfide, methane, ammonia, and sulfur dioxide. The sulfur plays a particularly interesting role when, in combination with hemolysis, it forms sulfhemoglobin, which is observed as a green discoloration of the skin. This colour change often begins around the lower abdomen, but as bacterial numbers increase rapidly and spread throughout blood vessels it causes them to appear much darker. The distinct coloration pattern resembles that of marble and so the phrase “marbling” was coined. In individuals who were suffering from bacterial infections at the time of death the increased numbers present in the body can also contribute to the putrefactive process (18).

Autolytic action spreads as the microorganisms, which are now colonizing the cadaver, migrate throughout the body. As the rate of autolysis increases the volume of gas also increases and causes the body, and most notably the stomach, to bloat and become distended (19). Blisters, known as bullae, also appear across the skin, filling with gases, decomposition fluids, or both. This buildup creates pressure within the tissues and the body as a whole causing gases to escape. Initially, this occurs through the body’s natural orifices or through injuries such as sharp force trauma, which may have been inflicted ante-, peri-, and post-mortem. In many cases the combination of increased pressure and degradation of tissue causes bonds between the dermis

and epidermis to break down. This causes skin slippage where skin sloughs off exposing the moist, pink basal layers beneath (20).

2.1.6 Active and advanced decay

As the integrity of the body is compromised, the decompositional fluids that are released into the surrounding environment, particularly soil, create a unique nutrient-rich cadaver decomposition island (CDI) around the body (21). The boundaries of the CDI will change with time as the purged leachate continues to spread away from the body laterally and vertically through the soil. In many cases the CDI environment is not initially conducive to plant life and can be phytotoxic to the local vegetation also because of the introduction of vast microbial populations. Although these biophysicochemical properties change over time, it can take up to a year for the vegetation to improve, hence the result of the CDI can persist even when the remains have been removed (22). This stage is also characterized by a significant surge in entomological activity with blow fly eggs hatching into larvae and additional species being drawn to the body to feed due to the increased surface area of the cadaver. The larvae mature and eventually migrate away from the body to pupate and this results in a second wave of adult flies that colonize the cadaver with further eggs lain to then hatch into larvae. This infestation and succession of numerous species consume the corpse, vastly increasing the rate of decomposition, and it is during this active stage that the biggest decrease in biomass occurs (23). Consequently, a significant amount of data is available on the rates and sequence of insect activity, which often prove useful in PMI determination. The advanced decay stage occurs when the body has lost most of its mass and has begun to dry. At this stage, entomological activity is

greatly reduced and fluids have either evaporated or drained from the body (24). The discontinuities in the skin have allowed for oxygen to be introduced, meaning that degradation by aerobic organisms, such as fungi, can begin. At this point an increase in certain chemicals, such as sodium, potassium, nitrogen, calcium, and magnesium, in the soil of the CDI is seen. Although it can be difficult to determine discrete points at which one stage of decomposition ends and the next begins, a surge in vegetative growth on the boarder of the CDI has been suggested as one such marker for the transition from decay to the dry remains stage (21).

In spite of increasing research in this area, many of the soil deposition processes associated with human cadaver decomposition are still not well understood and are dependent on local environmental factors (22). A major question revealed in the study conducted by Aitkenhead-Peterson *et al.* (25) is whether an endpoint, or return to baseline soil chemistry, occurs for cadaver decomposition islands. Expectations are typically that soil microbes cycle the nutrients and other compounds in the purge fluids where most are lost as volatiles with an eventual return of soil baseline chemistry. However, their study indicated that nutrients translocate further down the soil profile to depths exceeding the commonly sampled 5–7cm (25).

2.1.7 Skeletonization

The final stage of decomposition is the skeletal stage, which is the point at which all soft tissue has been fully decomposed and only the bones remain. Bones are a crystalline matrix formed of carbonated hydroxyapatite (HAP), which also contains collagen; this combination gives the bones their flexibility and strength. Ligaments, tendons, and periosteal tags will initially remain

adhered to the bones but, often with the assistance of faunal activity, these too break down causing the skeleton to begin to disarticulate (26). The bones themselves will also begin to decompose through a process of diagenesis. In its simplest construct, diagenesis relates to any form of naturally occurring bone modification that may impact on its structure, chemistry, or molecular content. Its focus is on three major factors: physical, chemical, and biological features. The latter is the one that has been studied the least (1). Several processes are at play when considering the breakdown of bone, namely, physical fracturing, degradation by microbes, dissolution in wet or acidic environments, and decalcification. The process of weathering was best summarized by Behrensmeyer (1978) (27) in her six-stage bone degradation scoring system, which is still used today.

This system focuses on the extent of cracking and splintering because of water lost through evaporation once decomposition of the protective soft tissue covering has occurred. Additionally, as temperature fluctuates throughout the day and night, the bone too will heat and cool. This can cause repeated expansion and contraction of the bone resulting in the formation of microfractures. Initially, the fresh bone will be ivory in color but as it is exposed to the sun it becomes bleached. Another impact on bone is the presence of vegetation. In subsurface remains, acid from plant roots can etch the bones as well as the root growth causing mechanical weathering to the skeleton. When bones are deposited in wet conditions, such as saturated soil or in water burials, certain mineral components can begin to disintegrate. Both soil and aquatic conditions will be discussed in detail later but one notable change is that, in acidic environments, HAP becomes soluble (28). Additionally, material can be taken into the bone from the surrounding environments due to the porosity of the bone. This can cause the

bone to darken as it takes on the color of the surrounding soil. In exposed remains, moss or lichen can grow on the surface; the impact this has on erosion is currently unknown. However, moss can cause staining on the bone.

3. Factors affecting the rate of decomposition

3.1 Clandestine burials

Burials are a common method chosen for the disposal of remains in homicide cases and many these are clandestine, or close to the surface. Like surface decomposition, the decomposing remains will have an impact on the surrounding soil, although a greater influence is seen on the body from the soil environment with subsurface burial. The combination of chemical, biological, and geological conditions in a location contributes to the characteristics of the burial environment and all subsequent postmortem changes (29). The first ever forensic study using cadavers was an experiment on the effects of subsurface burial at a range of depths from 1 to 4 ft (30). The study found that decomposition rates were generally slower than in surface deposits with an increased degree of preservation observed at greater depths. Currently, there is limited research and literature available on burial decomposition, particularly when looking at short-term interment, as it can be difficult to monitor changes accurately without repeated exhumation of the buried materials. This cycle of data collection would introduce the subject to air repeatedly and therefore not be reflective of protracted or permanent burial conditions. Also, it would disrupt the soil, fauna, and flora, which are known to reflect changes around

clandestine burials. Changes to surface vegetation have been recorded both in studies and anecdotal observations at crime scenes.

As described by Tibbett and Carter (2008) (29) while discussing CDIs, death of vegetation may also occur due to phytotoxicity with buried remains as leachates dispersed throughout the surrounding soil. However, as protein metabolism occurs in the corpse, ammonia leaches out and is converted to ammonium, which can be used by plants. Another feature of botanical change is the colonization of new and different plant species at the grave site where their seeds may have been exposed to more favorable conditions, either through the digging process or because of the change in soil alkalinity.

3.2 Temperature

Temperature has one of the most significant effects on the rate of decomposition. As it increases, the kinetic energy present and available for enzyme migration results in a greater chance of enzyme substrate collisions. Most enzymes will see an increase in action of 50%–100% with temperature increases of just 10°C. While all enzymes have an optimum functional temperature, they also have a wider threshold range within which their catabolic or metabolic activity occurs (31). Some deposition environments are not supportive of the traditional putrefactive decay and as a result a different process of decomposition is seen. If temperatures exceed specific thresholds, then enzymes may denature causing the cadaver to go into a state of arrested decay. Inversely, as temperatures decrease, energy levels also decrease, and bacteria and enzymes become dormant. For example, Micozzi and Pless (1997) (32) noted that bacterial growth is reduced significantly below 12°C and stops completely below 5°C.

In extremely cold conditions bodies may freeze, drastically altering the normal pattern of decomposition because the process of freezing alters the microstructures within the body. Specifically, a substantial proportion of the water is drawn out of the cells forming ice crystals at –5°C with the cytoplasm also affected if temperatures drop by a further 5°C. This causes the cells to either freeze internally or shrink. Overall, the process results in less intercellular diffusion and therefore the autolytic and putrefactive processes do not spread throughout the body. Freezing may also cause disruption to intercellular connections, which may cause softening and liquefaction upon thawing. Thawing begins in the external tissue cells and, unlike in remains that have never been frozen, decomposition begins superficially with internal organs remaining relatively preserved (33).

3.3 Mummification

Another extreme case of arrested decay is the natural mummification process where the preserved cadaver resists decay for a prolonged period while still maintaining features of its living appearance (34). The condition typically occurs in arid, hot, or dry environments. In locations such as chimneys, attics, basements, or other closed environments, mummification results in less destructive changes, which result in the desiccated tissue becoming significantly more preserved. Typically, the skin stretches tightly and becomes brittle with a dark leathery appearance believed to be caused by oxidation. Also, water is lost from the body causing internal organs to shrink parallel to an overall reduction in stature. As this type of decay is often a dry process, the pungent smells due to the release of gases and decompositional fluids during

putrefaction are not present. Therefore, this absence of a decomposition indicator can lead to an extended period between death and recovery.

3.4 Water burials

Water-based decomposition, with the marked presence of adipocere, cutis anserina, and skin maceration, creates postmortem profiles rarely presented with land-based decay. However, the presence of these characteristics on bodies recovered from water is not necessarily indicative of death in water, i.e., drowning. Specifically, cutis anserina is caused by rigor mortis in the erector pilae muscles of the dermis with the skin adopting a pimpled appearance like that of goose bumps. Additionally, the skin becomes wrinkled and swells, particularly in the extreme aspects of the limbs. The palms and soles show evidence of skin maceration or washerwoman's skin (35). When a body first enters the water, it will float due to the air trapped in the lungs or in clothing. Over time the air trapped within the body escapes and the remains begin to sink toward the bed of the water. Reflotation then occurs typically as gases build up during putrefaction. During the period of floating, parts of the cadaver will protrude above the water surface, causing different decomposition environments to occur within the same corpse. Although most of the body will stay submerged, it is an opportunity for insects or larger, nonaquatic scavengers to consume the remains (36). Like when bones became exposed on terrestrial surfaces, bone bleaching can occur in submerged remains as well. Very little is currently known about the timeframe for this process but it can be differentiated from sun bleaching by the lack of cracking and a more uniform coverage (37). Aquatic bleaching occurs because of chemical reactions, particularly in saline water. Typically, bones absorb sodium and

chlorine from the water, in the form of salts, when submerged for prolonged periods of time such as several years. As the bones dry, either due to being washed to the bank or because of recovery, the salt begins to form large crystals that cause fractures. It is important therefore that skeletal remains that are recovered from salt water environments are stored in similar salt water conditions until they are processed (38).

3.5 Scavenging

Although buried remains are mostly well protected from scavengers, increases in temperature result in soil drying and cracking, allowing volatile organic compounds (VOCs), the chemicals responsible for the distinctive smell associated with death and decomposition, to escape. These can then draw scavengers to the area. One of the greatest complications in the recovery and analysis of accessible remains is the impact of scavengers (39). Exposed soft tissue could become food for scavengers such as rats, birds, or different species of canids, particularly at times when other food sources are scarce (40). Although scavenging populations and species differ significantly around the world, an experiment conducted in the United Kingdom showed that, while the remains of rats were fully accessible to all species, the only animals to feed on the cadavers were foxes, crows, and magpies (41). This may be because of the small body size of the analog in comparison to human remains, and the unlikely occurrence of cannibalism by scavenging rats.

Larger, carnivorous predators are known to disarticulate bodies and transport sections of remains, often limbs, away from the initial deposition or burial sites. Here, the knowledge of

the forensic taphonomist on feeding and dispersal patterns of native scavengers is invaluable in the coordination of any search and recovery efforts. A large proportion of this disarticulation and scattering of remains is due to the feeding preferences of the animals, the presence of other, larger scavengers, and simple anatomical considerations. Some scavengers have teeth and claws better adapted for cutting through flesh, while others are simply larger and stronger, allowing them to drag or carry remains. This can, however, leave trailing evidence, such as decompositional fluids emitting VOCs, across the ground surface, which can aid in the search and recovery process (42).

3.6 Trauma and associated material

Open wounds or cuts in a cadaver have been reported to promote its decomposition rate (29, 43, 44) since they serve as entry points for external microorganisms and insects. The presence of clothing on a cadaver has been reported to aid the rate of decomposition. For example, maggots tend to avoid sunlight by gathering under clothing (43-45).

4. Clandestine gravesites in Australia

Clandestine gravesites are prevalent throughout every state in Australia and there are numerous articles detailing the presence of these sites, be it Aboriginal burials or graves relating to unlawful homicide.

4.1 Methods employed in excavating clandestine graves

4.1.1 Arbitrary Level Excavation

Literature by multiple authors argue that graves should be excavated using the ALE method. ALE, also referred to as the Pedestal method (46), is the standard method routinely used during forensic death investigations for the recovery of individual buried remains (46). It is a common method used in traditional archaeological assessments that has been widely adopted in forensic investigations (46). During the arbitrary level excavation of a grave, soil is removed in a succession of predetermined levels, usually 0.05 m, 0.10 m, or 0.20 m in depth (47), over an arbitrary but carefully measured area, usually determined by the perceived size of the grave at surface level. As evidence is identified the earth 'matrix' that surrounds it is removed leaving each item upon a soil 'pedestal'. These items are measured in situ and only removed when they are deemed to be hindering the progress of the excavation (48-51). During this process, soils that comprise the deposits backfilling the grave as well as the surrounding natural/man-made strata through which the grave was originally dug are removed in spits across the defined area of excavation. In order to provide access to the burial, trenches are often dug around the remains resulting in the removal of the grave walls (48, 52, 53). Some practitioners advocate against the removal of the grave walls however, as these surfaces may be of assistance when interpreting the method by which the grave was constructed, and assist investigators in establishing links between the crime scene and the perpetrator(s) (26, 47, 51, 54)

One of the benefits of utilising the ALE method is how easy it is. Because spits can be easily and accurately measured to predetermined depths, less archaeological experience is required. This

allows forensic investigators who have significantly less archaeological expertise to successfully excavate clandestine grave sites. Spennemann and Franke (55) found that ALE allowed for spatial and depth control for both soil removal and artefact recovery when exhuming six caskets. Additional benefits to the ALE method proposed by Tuller and Duric (46) include: easier access and viewing of remains and evidence; ability to take dynamic photographs for a strong visual impression of the remains and evidence; reduces the amount of time spent standing on top of the remains during excavation (47), which may damage the remains or evidence (46); and the use of trenches assists with potential water drainage issues that can damage the remains and evidence (46, 47).

The arbitrary level excavation method has several perceived advantages, including: spatial and depth control of soil removal and artefact recovery; easier access to the remains and artefacts from different angles; dynamic photographs can be taken of both the human remains and artefacts; it assists with potential water drainage issues that can damage the integrity of the grave structure; and it limits the time spent standing on a grave structure of limited size that could damage the human remains and artefacts (46, 53-56). Notionally less archaeological skill and experience are required to utilise this method, as spits can be easily measured and levelled to accurate standard depths. However, there are inherent problems with this method, including: the method destroys and ignores stratigraphic interfaces and layers present within the grave; it introduces artificial divisions of deposits and evidence which can result in evidence retrieved during the process of an excavation having no known stratigraphic origin; it results in the mixing of strata and artefacts from the grave structure (fills and cuts) and natural strata

through which the grave was dug potentially leading to contamination of soils and artefacts that may pre or post-date the grave; the grave walls can only be recorded in plan at the interface of each arbitrary level (if distinguishable from the natural strata) which will not always allow for the accurate recording of the grave cut including tool marks; and pedestalled artefacts may be moved during excavation (57-62).

Despite these weaknesses, the arbitrary level excavation method continues to have advocates for its application; it has been argued that this is largely due to the fact that, normally, graves lack complex stratigraphy and are usually comprised of a singular fill, and therefore the application of arbitrary units is justifiable. The primary emphasis when utilising this method is often upon the recovery of artefacts and human remains, rather than understanding the entirety of the grave formation process. Arbitrary level excavation provides the easiest and most efficient method for meeting this objective (56, 63).

4.1.2 Stratigraphic Excavation

When using this method, separate archaeological stratigraphic contexts are identified and excavated individually in sequence, and recorded as individual stratigraphic phenomena. The entire grave is viewed as an archaeological feature. Thus the fills and interfaces are normally revealed and recorded in their entirety and grave walls may be exposed and maintained throughout the entire excavation process. This allows for the retention of tool marks and geotaphonomic evidence present on the surfaces of the grave walls and grave floor (54). here are several perceived advantages to stratigraphic excavation including: three dimensional

recognition, assessment and recording of each stratigraphic context; revealing of interfaces between deposits; chronological recovery of evidence by context; spatial and depth control of soil removal and artefact recovery; prevention of contamination between stratigraphic contexts; dynamic photographs can be taken of both the human remains and artefacts reflecting their chronological deposition; and removal of deposits that records the sequence of deposition to aid in the reconstruction of events (64). The main problems with this method are: without tents and other precautions water can collect in the grave; that excavation in limited spaces and at depth can limit access to the human remains (46); difficulties in recognising individual stratigraphic contexts, especially interfaces; that the method is more complicated to perform than other methods; and that the method may be perceived to slow down excavation.

The main benefit of utilising the SE method is the ability to interpret events through reconstruction of the depositional sequence of soil types and evidence (46). Three dimensional documentation of each stratigraphic context (46) allows for the soil and associated evidence to be interpreted within the grave site (26) Reconstructing the sequence of events during the burial is crucial to forensic investigations. Spenneman and Franke (55) were able to interpret the events that occurred during burial due to information gathered surrounding the stratigraphic context of the grave fill. Additional benefits proposed by Evis *et al.* (64) include: revealing interfaces between each deposit; spatial and depth control for both evidence recovery and removal of soil; prevention of contamination between different soil stratigraphy due to the excavation of separate stratigraphic contexts; chronological recovery of evidence which allows for the interpretation of the events that occurred at, or around, the time of burial;

and the ability to take dynamic photographs of the remains and evidence which reflect the order of deposition. Tuller and Duric (46) also found that SE is better at maintaining the grave contents in situ, there is greater control over the excavation process, and the grave formation process can be better understood, all factors that are vital in forensic investigations.

4.1.3 Combined approach ALE/SE

Journal articles by multiple authors and textbook publications by Dupras *et al.* (26) and others outline a combined ALE and SE method. This combined methodology is based on three basic principles obtained from the ALE and/or SE methods: utilising a grid for horizontal control vertical control using excavation units (XUs) responsive to stratigraphic contexts; and a final reconstruction of three-dimensional stratigraphy post-excavation. This was the beginning of the 'combined excavation approach' routinely used today in forensic investigations for clandestine grave site recovery. Essentially, this approach involves the removal of soil in arbitrary levels, whilst maintaining the integrity of the grave walls. When utilising the combined approach, graves should be dug in defined arbitrary levels of 1 inch (64) or 2 inch spits (46). The combined approach is designed to maximise evidence recovery (26) and increase the interpretation ability of the associated evidence. Theoretically, the combined excavation approach allows for increased evidence recovery rates, recovery of tool marks and trace evidence from the grave walls and floor, and recovery and interpretation of stratigraphic contexts. In addition, this method can be applied by individuals, such as crime scene officers, that have limited knowledge in identifying soil stratigraphy, without losing vital stratigraphic evidence. Despite the

theoretical benefits, there is no scientific literature that evaluates the practicality, suitability, or validity of utilising the combined ALE and SE approach (64).

4.1.4 Ground Penetrating Radar

GPR systems usually consist of a signal generator, transmitting and receiving antennae, and a monitor that can display real-time data of the subsurface being imaged (65, 66). The transmitting antenna emits electromagnetic (EM) waves into the ground, which are then reflected to the receiving antenna as they encounter subsurface features with different dielectric properties. Dielectric permittivity, which represents the degree of polarisation a material experiences under the influence of an external electric field and is conceptually very similar to conductivity, is principally controlled for subsurface materials by composition, water content, and porosity. During a GPR survey, the EM waves will reflect back to the receiver at interfaces between different materials, such as changes in lithology, boundaries between natural and anthropogenic materials, and voids. The greater the contrast in dielectric permittivity between these materials, the higher amplitude the GPR response. The reflections are recorded in a one-dimensional (1D) plot called a trace, which can then be combined with other traces to create a 2D profile, 3D cubes, and/or amplitude slice maps. The receiving antenna also measures the time that the signal takes to return to the antenna and so, in combination with an estimation of the velocity properties of the subsurface, can provide an estimate of the depth of features of interest (67).

The antennae frequency used during data collection is important because it affects both the depth of penetration and the vertical resolution of the signal. GPR antennae exist in a wide range of frequencies, ranging from~50MHz to ~2000MHz (66). Lower frequency antennae allow for a greater depth of penetration but have a lower resolution than higher frequency antennae. Most forensic and archaeological investigations for the detection of unmarked graves are undertaken using antennae in the range of 250–900 MHz. Technological developments have allowed for multi-frequency antennae, which provide multiple frequency data from the same survey, providing both high resolution and deeply penetrating outputs simultaneously (68). Another recent development is the use of 'hyperstacking', in which each trace is collected many times and summed together to improve the signal-to-noise ratio, thus improving dynamic range limitations. While frequency and other approaches can assist with obtaining a greater depth of penetration, this can still be limited by conductive materials and is dependent on moisture levels. This limitation is somewhat ameliorated regarding the detection of unmarked graves because the most obvious physical and lithological evidence of soil disturbance is usually found in the upper 10s of centimetres of the subsurface, regardless of the depth of the burial (67)

Another important factor to consider when performing a GPR survey is the trace increment and line spacing. To ensure that all targets are located, the line spacing should be no greater than half the shortest possible axis of the target, which when locating graves of adults is usually no more than 0.5m. Naturally, a higher line spacing results in high resolution data, but also increases the survey time. This has been recently improved with the introduction of 3D GPR antennas (one antenna casing that contains multiple transmitters and receivers

which provides high density 3D data and can be acquired using a motorised survey platform) (68)

4.2 Climate in Australia

Australia's climate is governed mostly by its size and by the hot, sinking air of the subtropical high-pressure belt (subtropical ridge). This moves north-west and north-east with the seasons. The climate is variable, with frequent droughts lasting several seasons, thought to be caused in part by the El Niño-Southern Oscillation. Australia has a wide variety of climates due to its large geographical size. The largest part of Australia is desert or semi-arid. Only the south-east and south-west corners have a temperate climate and moderately fertile soil. The northern part of the country has a tropical climate, varying between grasslands and desert. Australia holds many heat-related records: the continent has the hottest extended region year-round, the areas with the hottest summer climate, and the highest sunshine duration (69).

Because of its elevation (650 m (2,130 ft)) and distance from the coast, the Australian Capital Territory experiences a dry, continental climate. Canberra has warm to hot, dry summers with heat waves. Canberra has cool to cold winters with occasional fog and frequent frosts. Many of the higher mountains in the territory's south-west are snow-covered for part of the winter. Thunderstorms can occur between October and March, and annual rainfall is 623 mm (25 in), with rainfall highest in spring and summer and lowest in winter (69).

Over half of New South Wales has an arid or semi-arid climate. The eastern portion has a temperate climate, ranging from humid subtropical from its northern border to the Central Coast and most of Sydney, and oceanic to the south coast. The Snowy Mountains region in the south-east falls in the alpine climate or subpolar oceanic climate zone, with cool to cold weather all year around and snowfalls in the winter. Further inland, the climate is semi-arid and a desert climate towards the western part of the state (69).

The weather in the southern half of the state is generally warm to hot in summer and cool in the winter. The seasons are more defined in the southern half of the state, especially in the South West Slopes, Central West and the Riverina regions. Rainfall usually peaks in the summer in most of parts of the state, though the Riverina region, which is in the southern-central part of the state, bordering Victoria, has drier summers and a winter rainfall peak. On a hot summer day, a southerly buster may at times moderate the extreme heat experienced in the coastal New South Wales region, from Port Macquarie southwards to Nowra (69).

The warmest region is the north-west, where summers are very hot, and winters cooler and drier. The weather in the northeast region of the state, or the North Coast, bordering Queensland, is hot and humid in the summer, with a rainfall peak, and mild in winter with more sunshine, and little seasonal temperature difference. The Northern Tablelands have relatively mild summers and cold winters, due to their high elevation in the Great Dividing Range. The southeast coastal plain, which lies on the leeward side of the Great Dividing Range, experiences foehn winds, particularly between winter and spring, which can elevate fire danger.

The coldest region is the Snowy Mountains where the snow and frost continues for a long period during the winter months. The Blue Mountains, Southern Tablelands and Central Tablelands, which are situated on the Great Dividing Range, have mild to warm summers and cold winters, although not as severe as those in the Snowy Mountains. Some areas situated in or around the range, such as Bathurst, Goulburn and Bowral, among other places, have recorded freezing and/or near-freezing lows in most months of the year, unlike other places of similar latitude and altitude in the northern hemisphere (69).

The Northern Territory has two distinctive climate zones. The northern end, including Darwin, has a tropical savannah climate (Köppen Aw) with high humidity and two seasons, the wet (October to April) and dry season (May to September). During the dry season nearly every day is warm and sunny, and afternoon humidity averages around 30%. There is very little rainfall between May and September. In the coolest months of June and July, the daily minimum temperature may dip as low as 14 °C (57 °F), but very rarely lower, and frost has never been recorded (69).

Because of its size, there is significant variation in climate across Queensland. Low rainfall and hot summers are typical for the inland west, a monsoonal 'wet' season in the far north, and warm subtropical conditions along the coastal strip. Inland and in southern ranges cooler temperatures are experienced, especially at nights. The climate of the coastal strip is influenced by warm ocean waters, keeping the region free from extremes of temperature and providing moisture for rainfall (69).

The majority of South Australia has the arid and semi-arid climates. The southern coastal parts of the state have a Mediterranean climate with mild wet winters and hot dry summers. The highest rainfall occurs along the southern coasts and the Mount Lofty Ranges (with an average annual rainfall of 1,200 millimetres (47 in) in the vicinity of Mount Lofty); the lowest rainfall occurs in the Lake Eyre basin where the average annual totals are less than 150 millimetres (6 in) and possibly even 100 millimetres (4 in). Most of the rain in the southern districts of the State fall during the winter months when the sub-tropical high-pressure belt is displaced to the north over the Australian continent (69).

Tasmania has a cool temperate climate, with most areas under an oceanic climate, with four distinct seasons. Summer lasts from December to February when the average maximum sea temperature is 21 °C (70 °F) and inland areas around Launceston reach 24 °C (75 °F). Other inland areas are much cooler; Liawenee, located on the Central Plateau, is one of the coldest places in Australia with February temperatures ranging between 4 to 17 °C (39 to 63 °F). Autumn lasts between March and May and experiences changeable weather, where summer weather patterns gradually take on the shape of winter patterns (69)

Victoria has a varied climate despite its small size. It ranges from semi-arid and hot in the north-west, to temperate and cool along the coast. Victoria's main land feature, the Great Dividing Range, produces a cooler, mountain climate in the centre of the state.

The coastal plain south of the Great Dividing Range has Victoria's mildest climate. Air from the Southern Ocean helps reduce the heat of summer and the cold of winter. Melbourne and other large cities are located in this temperate region.

The Mallee and upper Wimmera are Victoria's warmest regions with hot winds blowing from nearby deserts. Average temperatures top 30 °C (86 °F) during summer and 15 °C (59 °F) in winter. Victoria's highest maximum temperature of 48.8 °C (119.8 °F) was recorded in Hopetoun on 7 February 2009, during the 2009 south-eastern Australia heat wave. A screen temperature of 50.7 °C (123.3 °F) was recorded on 7 January 1906 in Mildura (69).

Most of Western Australia has a hot arid and semi-arid climate. However, the south-west corner of the state has a Mediterranean climate.[73] The area was originally heavily forested, including large stands of the karri, one of the world's tallest trees. This agricultural region of Western Australia is in the top nine terrestrial habitats for terrestrial biodiversity, with a higher proportion of endemic species than most other equivalent regions. Due to the offshore Leeuwin Current, the area numbers in the top six regions for marine biodiversity, containing the most southerly coral reefs in the world (69).

Average annual rainfall varies from 300 mm (12 in) at the edge of the Wheatbelt region to 1,400 mm (55 in) in the wettest areas near Northcliffe, the southwesternmost tip of Australia, but in the months of November to March, although rain still falls, evaporation exceeds rainfall and it is generally very dry. Plants must be adapted to this as well as the extreme poverty of all soils. A major reduction in rainfall has been observed, with a greater number of rainfall events

in the summer months. The central four-fifths of the state is semi-arid or desert and is lightly inhabited with the only significant activity being mining. Annual rainfall averages about 200 to 250 mm (8 to 10 in), most of which occurs in sporadic torrential falls related to cyclone events in summer months (69).

4.2.1 Climate and decomposition – what are the effects?

In general, the climate of Australia can be described as variable, with the largest part of Australia being desert or semi-arid. Temperature has one of the most significant effects on the rate of decomposition. As it increases, the kinetic energy present and available for enzyme migration results in a greater chance of enzyme substrate collisions. Most enzymes will see an increase in action of 50%–100% with temperature increases of just 10°C. While all enzymes have an optimum functional temperature, they also have a wider threshold range within which their catabolic or metabolic activity occurs (31). As the temperature increases in different parts of Australia, it is logical to assume that decomposition speeds will also increase in the hotter parts of Australia. Conversely, decomposition will tend to slow down in the colder regions of the country. Mummification also has the potential to occur in the hot, dry, and arid parts of the country, where the the skin stretches tightly and becomes brittle with a dark leathery appearance believed to be caused by oxidation. Also, water is lost from the body causing internal organs to shrink parallel to an overall reduction in stature (34).

Desiccation results from the dehydration of soft tissues with the skin eventually becoming dark, dry, and leathery. It affects body parts which are generally exposed to either airflow or dry

aerated burial conditions as they allow rapid drying of the soft tissues limiting putrefaction by destructive micro-organisms. Within a stable environment a desiccated body can remain preserved for years (34, 70-75). Adipocere formation occurs by alteration of subcutaneous fat of a corpse into a grey-white lipid mixture. With time, this substance becomes a hard, brittle shell, which retards decomposition (70, 76, 77). The chemical processes of adipocere formation are largely understood, with the basic process resulting from the hydrolysis and hydrogenation of adipose fats. Extensive studies regarding its chemical composition demonstrate that adipocere consists predominantly of saturated fatty acids, namely myristic, palmitic and stearic acids, with lesser amounts of hydroxyl and oxo-fatty acids (77, 78). Like desiccation, adipocere can persist for years if the environment is stable. However, it is not necessarily a stable product, with certain conditions inducing further decomposition.

Desiccation and adipocere are usually presented as contrasting conditions. However, slight adipocere formation is common in desiccation. The two are related in that the utilisation of the internal body water can be sufficient to hydrolyse the fat and in turn help to dehydrate the tissues. Dehydration and desiccation will accompany adipocere formation in bodies where little or no exogenous water is available (72, 76, 78-80).

This is called the atypical condition for adipocere because most reported instances of adipocere are associated with conditions such as water immersion, wet graves or damp vaults (80). Nevertheless, in temperate climates, differential decomposition due to the combination of decay, desiccation and adipocere is not often described. One published case describes a body

on a beach in Southwest Scotland. The body was discovered 8 months after disappearance. It was partly decomposed, partly mummified and showed a small degree of adipocere (81).

4.2.2 Legal resources for clandestine grave excavation in Australia

Federally, the main law enforcement agency is the Australian Federal Police (AFP), which has a wide mandate to enforce Australian criminal law and protect its national interests.

All states in Australia have a functioning state police service consisting of general duty policemen and women and also specialized sections of the service e.g. canine, air wing, forensic police.

5. Other animals as a model for graves/human forensic studies

In many experimental studies, animals are used as analogues when human remains are either unavailable or unlicensed. At research facilities across the globe, such as the Wrexham Glyndŵr University's Forensic Science and Crime Scene Research Area, other easily accessible mammals are used in the place of human cadavers. Most research is commonly conducted on pigs, rabbits, mice, or rats, either dealing with the carcass as a whole or select anatomic sections, for example, trotters. The choice of these is often dependent on the cost, availability, and scale of the experiment (82). Turner and Wiltshire (83), Notter *et al.* (84) and Stokes *et al.* (82) have all recorded disparities in outcomes from the use of pigs as human analogues with the latter revealing that none of the analogues utilized had similar results when measuring decomposition to those obtained when testing on human remains in a subsurface soil environment. Research looking at the use of pigs and rabbits in comparison to human cadavers

across different seasons has showed that their use as analogues does not yield the transferable results originally anticipated (85).

The research, which was presented to the American Academy of Forensic Science, summarized that the decomposition of pigs was quicker than that of both humans and rabbits in spring and summer. Initially, rabbit decomposition was the slowest overall until the point of peak maggot activity, which showed significant progression over a 24-h period. The results of the winter study shed light on the scavenging preferences of local wildlife with human remains being consumed initially. Finally, one of the most notable conclusions was the degree of variation within the human subject group. Both the rates of pig and rabbit decomposition were consistent within the samples, while human remains decomposition proved much more variable and therefore harder to predict.

5.1 Ethical and legal factors associated with the use of human models

Many ethical issues are raised when considering the use of human remains for research purposes. Thus any experimentation that makes use of cadavers must be highly regulated (82). Existing legislation in the United Kingdom prevents any form of taphonomic experimentation to be performed on human tissue, as governed by the Human Tissue Authority (HTA), hence there is a significant push from academics in the field to begin discussions with the HTA surrounding the current legislation. In Australia, if researchers intend to import human remains into the country, A written declaration or the permission of a Commonwealth Human Biosecurity Officer

(HBO) will be required for the remains to be brought into Australia. The written declaration must be from the person bringing in the body or body part and must state:

- that the body or part of the body has been donated for scientific or research purposes; and either:
- if the person is aware that the body or part of the body has, or had before death, signs or symptoms of a listed human disease and the name of the listed human disease; OR
- that, as far as the person is aware, the body or part of the body does not have, or did not before death have, any signs or symptoms of a listed human disease (86)

As well as the legal issues, previous attempts to establish human research facilities for forensic research in the United Kingdom have been met with several objections. One of the most notable attempts to establish a facility was in 2010 when Omega Supplies Ltd. put forward funds to support a facility in Lincolnshire (87). However, concerns over location and availability of cadavers were raised as well as a lack of public support for the proposal. To mitigate for the last issue, public opinion surveys have been launched to better inform UK citizens and address their worries and concerns. For example, research from Staffordshire University in 2014 revealed an overwhelming recognition from the public that there is a need for a UK-based human research facility, with 92% agreeing that a centre should be established (88).

5.2 Discrepancy of using animals in observing human decomposition

Taphonomic studies are often designed using animal analogues to estimate the processes that a human cadaver would undergo in specific environmental conditions (89-98). These models are

required due to the scarcity of available human cadavers and more importantly because of the ethical and cultural constraints that restrict their use. Decomposition trials use a range of different mammalian cadavers or their skeletal muscle tissue (SMT) as analogues for humans including pigs (91, 94, 95, 99-102), sheep (103-106) dogs (90) rats (93, 107) guinea pigs (89) deer (108) bison (108) and rabbits (96, 109, 110) Larger mammals are preferred for many surface decomposition studies, due to the accelerated rate of decay in smaller analogues and difficulties associated with identifying each stage of decomposition (91). Analogues are used to investigate a range of parameters such as insect succession (90-92, 111, 112), decomposition chemistry (95, 100-102, 113, 114), microbial activity (94, 103, 105, 115), and general decomposition processes (90, 91, 96). To date, there has been minimal investigation into the appropriateness of using animals as models for human cadavers in decomposition trials. Two studies have been carried out comparing pig cadavers with human cadavers and comparing pig cadavers of several different sizes with human cadavers and analyzing the insects that colonized the bodies during decomposition (97). Of the insects captured at the pig and human cadavers, 99.67% were common to both species, with only very rare insects being found exclusively at either one. Thus, there was no apparent preference for either cadaver species based on the entomological and statistical analysis (97, 116).

Reed (90) created an insect succession list based on the decomposition of 43 canine cadavers. These data were compared with insect succession in human cadavers (117). An overlap in the insects of forensic interest associated with both cadaver types was observed. While there is an apparent similarity in the insects colonizing human cadavers and two mammalian sources

(porcine and canine), there are little available data for making a comparison with other mammals. In a later study, Mann *et al.* (44) acknowledged that decomposition trials using dog carcasses (20–40 kg) were not comparable with human cadavers (50–65 kg) as the dogs decomposed more rapidly. The only observed similarity was faster decomposition of head regions in both cadaver species, likely related to the facial orifices that attract increased insect activity.

A further limitation with the current literature is that the majority of data are obtained for surface decomposition events (89-93, 96, 97, 116, 117) and very little data are available for buried cadavers (94, 95)or cadaver analogues (100-104, 113). This is often related to the exclusive investigation of entomology associated with cadaver decomposition which typically requires surface exposure. Hence, burial environments are markedly under investigated in the sphere of forensic taphonomy.

5.3 The use of pigs as an analogue for human decomposition

The origin of pigs as human proxy can primarily be traced to entomological studies where the use of animal proxies for the comparison of insect succession throughout decomposition is well tested. Rodriguez and Bass (117) compared available insect succession data collected from canine cadavers (90) to that observed on human cadavers and found significant overlap. It appears that most insect species (99.67%) show no preference between pigs or humans (97, 118). However, caution is warranted when using entomological studies to validate the use of pig carrion in decomposition studies. While the entomologist and anthropologist both seek to

understand PMI, it is incorrect to assume that the disciplines are studying the same underlying physiological phenomenon. The entomologist is concerned with insect development, progression, and succession guided by the sum of the decomposition event (e.g., the release of chemical constituents that guide chemotaxis, moisture content and related patterns of oviposition, and visual and mechanosensory cues that govern insect movement). Conversely, the anthropologist is concerned with the sum of stages of gross morphological change, which may correlate with various stages of decomposition (e.g., rate, chronology, and presentation of tissue dissolution under various circumstances). Therefore, the biological features that make pigs a viable research tool in entomology cannot be assumed to translate to taphonomic investigation. Wang *et al.* (119) conducted a comparative analysis of insect succession on human remains, pig carrion comprising two size categories, and rabbit carrion. They conclude that body size and decomposition rate share an inverse relationship, and insect species diversity and complexity are more complicated on larger remains. Dipteran development rate was consistent across all size and species categories, although a subset of forensically significant *Calliphoridae* species were unable to complete first-generation development on rabbit carrion. Dautartis *et al.* (120) observed a similar correlation between body size and trajectory of decomposition. Five each of pigs, humans, and rabbits were used to study gross morphological changes and insect activity across a 5-month interval. The conclusion was that the pattern of decomposition differed between the three groups, and they were not likely to be interchangeable for decomposition research. However, the continuing challenge in using human remains for research study is sample size. While these studies present intriguing patterns, there is still a need for research conducted within human samples, using larger

sample sizes, and in differing environments. Additionally, longitudinal studies are warranted in dry areas where desiccation occurs, and bodies take much longer than 5 months to skeletonize.

Research done by Connor *et al.* (121) found that in approximately 60% of the pig specimens, the intestines ruptured through the abdomen during the bloat phase; abdominal rupture did not occur in any of the human specimens. While humans and pigs present anatomical, physiological, and genetic overlap, fundamental differences between the two species exist. While both humans and pigs are monogastric, key differences in the structure and function of the gastrointestinal tract (GIT) may affect the rate, timing, and trajectory of decomposition. On average, the human intestine (large and small) is 7.5 m in length, as opposed to 23 m in the pig. The pig small intestine further presents differences in segment length and branching of mesenteric vessels and attendant anastomoses (which facilitate cellular communication with surrounding tissues). In the human GIT, ileal Peyer's patches (PP) are an aggregate of lymphoid modules that facilitate an immune response within the mucosa by monitoring intestinal bacteria and inhibiting growth and spread of pathogenic strains. Conversely, the ileal PP in pigs is continuous, which not only constitutes a structural difference, but also implies major differences in typical bacterial load, potential exposure to pathogenic bacteria, and enteric colonization. A pyloric diverticulum—a site for microbial metabolism of ingesta—is present in pigs but absent in typical human anatomy (122). Finally, the cecum, ascending and transverse colon, and the proximal portion of the descending colon are oriented in a series of centrifugal and centripetal coils to accommodate length and orientation associated with quadrupedalism, which differ from the folded and “stacked” structure of the human intestines.

The sum of these many subtle differences may contribute dramatically to gross differences in decomposition, such as the abdominal rupture observed in this sample. In more basic terms, while this phenomenon is likely multifactorial, a significantly longer GIT summarily constitutes larger cubic volume of lumina within which decompositional gasses have the potential to aggregate, resulting in greater potential for eruptive response.

Overall similarity in total body scores (TBS) in pigs and humans in early decomposition may be an artifact of the overfitting of data to the scoring system; data collection necessitates that a score be applied so the "best choice" category regardless of overall technical accuracy. Only six categories in the head/neck and five in the trunk and limbs describe early decomposition. Limited scoring options may reduce or eliminate the resolution necessary to highlight divergence in gross change. Keogh *et al.* (123) used the Megyesi *et al.* (124) TBS to define the typical trajectory of human decomposition and compared this to observations made within a sample of 20 pig carrion. The authors concluded that the gross changes observed between the two groups were sufficiently different to warrant a separate scoring system for pig carrion. However, TBS comparison was limited to pigs and did not include humans in the sample environment, so the differences observed may be a combination of species and environment. Total body score values for both species plateaued between 21 and 24 for an extended period of ADD. The humans plateaued in moist decomposition for a longer period of ADD. The pigs were a clinically healthy weight, while over half the human sample was overweight or obese. Body fat does impact decomposition, hindering dissipation of heat and providing liquid for

bacterial growth and insect oviposition. With regard to the human sample, the presence of diabetes mellitus accelerates decomposition (45).

When compared using the TBS model, the trajectory of decomposition observed between human and pig samples diverged in rate and gross presentation. Additionally, the TBS scoring model did not perform well in the arid environment at the Forensic Investigation Research Station, Colorado (FIRS). Regional decomposition patterns may require regional scoring models. Nonhuman proxies do provide a superficially homogenous sample allowing isolation of individual variables and have the potential to indicate trends in taphonomy. Human samples tend to be more variable, particularly in body composition and cause of death, both of which affect the pattern of decomposition. Pigs may be useful in studying general trends. However, they are not a substitute for human subjects, and caution is warranted when attempting to apply data derived from pigs to human subjects, especially in medicolegal investigation. Above all, reliance on a relatively homogenous proxy sample may make researchers overconfident in their ability to predict the timing and patterns of decomposition. The TBS model used by many decomposition studies does not work well with pigs and, for the data presented, fit neither pig nor human data in late-stage decomposition. This was likely due to the desiccation seen in FIRS' semi-arid environment and highlights the need for region-specific validation of models used to estimate post-mortem interval.

Further research done by Stokes *et al.* (82) compared the decomposition dynamics of mammalian analogues in experimental taphonomy. The purpose of this study was to investigate the decomposition of human and other mammalian skeletal muscle tissue (SMT) in

a soil environment to determine the potential of animal analogues to be used as models for human SMT in decomposition studies. The authors were interested to see whether decomposition rates, microbial activity, and changes to soil chemistry (including nutrient release) differed between mammals whose muscle tissues were easily available for taphonomic research. In our experiment, we chose to compare porcine, ovine, and bovine SMTs with human SMT using established experimental techniques.

Measurements were carried out on detritosphere soil and included pH, potassium, phosphate, ammonium, and nitrate. Microbial activity was also monitored over the course of the study. In a clandestine grave, a decomposing cadaver is in intimate contact with the surrounding environment, commonly soil. Soil is composed of many different components: these include organic material from plants and animals, mineral matter, and a living component of macro, meso, and micro faunal populations (98, 125, 126). These components are interlinked, and changes to one aspect will generally lead to variations in the others. However, quantification of these variations can be difficult. The decomposer subsystem of the terrestrial environment has two major functions: mineralization of essential elements and formation of soil organic matter (127). A cadaver is a rich source of both essential elements and organic molecules and therefore becomes a hot spot for biotic and abiotic transformations (21, 98, 125).

Stokes *et al.* (82) found that no single SMT was an ideal predictor of human SMT for soil decomposition studies. Several measurements differed significantly between porcine, bovine, ovine, and human SMTs, including microbial activity, gravesoil chemistry, and nutrient

concentrations. Microbial activity in soil exposed to porcine and bovine tissue was higher than in soil exposed to human tissue. The concentrations of the nutrients PO_4^-, K^+, and $NH4^+$ in detritosphere soil surrounding nonhuman tissue were often significantly higher than those in soil surrounding human tissue. Nonetheless, the overall patterns of nutrient fluxes and chemical changes in nonhuman SMT followed that of human SMT. Ovine tissue was the most like human tissue in many measured parameters. Therefore, no nonhuman tissue types were precise predictors for human tissues in taphonomic studies, but all offered some approximation in decomposition dynamics. Gravesoil pH was related to $NH4^+$ concentration. Differences in soil pH surrounding the different tissue types corresponded to differences in $NH4^+$ concentrations for each SMT source. However, the relationship between pH and $NH4^+$ concentration appeared to vary between each tissue source as demonstrated by the R^2 values. This may be linked with variations in the nutrient compositions of SMT from different mammalian sources, further explaining the variations observed in other nutrients measured. Nutrient compositions vary not only between different species, but also breed, sex, age, location, and feed (128-131). Bone density of different animal species also show variation (132).

The results of this study showed many differences between porcine, bovine, and ovine SMTs when considering them as analogues for human SMTs in decomposition soil studies. However, there were also similarities observed for each species when compared to human SMT. Porcine, ovine, and bovine SMT had points of no significant difference from human SMT in most measures carried out over the course of the study. Lamb SMTs appeared to be the most similar to human SMTs in pH and nitrate, while porcine and human SMTs were most similar based on

eletroconductivity. Based on this evidence, there is cause to still consider the use of SMT from other species as models for human SMT in taphonomic studies. However, care must be taken in interpreting the results.

6. Conclusion

With further research, it may be possible in the future to use animal models as accurate analogues for human cadavers to measure specific measurements. For example, pigs have shown similar insect successions to humans (97), and insects of forensic interest are also common to dogs (90). It may become possible to tailor decomposition studies using animal models to more precisely mimic specific artifacts of human decomposition.

7. References

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Part Two
Manuscript

A REVIEW OF THE USE OF *SUS SCROFA* AS AN ANALOGUE TO HUMAN DECOMPOSITION STUDIES

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1. Abstract

Taphonomic studies are often designed using animal analogues to estimate the processes that a human cadaver would undergo in specific environmental conditions. These models are required due to the scarcity of available human cadavers and more importantly because of the ethical and cultural constraints that restrict their use. Decomposition trials use a range of different mammalian cadavers or their skeletal muscle tissue (SMT) as analogues for humans including pigs, sheep, dogs, rats, guinea pigs, deer, bison, and rabbits. Larger mammals are preferred for many surface decomposition studies, due to the accelerated rate of decay in smaller analogues and difficulties associated with identifying each stage of decomposition. Analogues are used to investigate a range of parameters such as insect succession, decomposition chemistry, microbial activity, and general decomposition processes. . Two studies have been carried out comparing pig cadavers with human cadavers and comparing pig cadavers of several different sizes with human cadavers and analyzing the insects that colonized the bodies during decomposition. Of the insects captured at the pig and human cadavers, 99.67% were common to both species, with only very rare insects being found exclusively at either one. Thus, there was no apparent preference for either cadaver species based on the entomological and statistical analysis. The use of nonhuman animal proxies for human remains in decomposition studies has been based primarily on the assumption that most carrion, especially those that are similar in size and integument, will undergo the same decomposition process at an analogous rate as humans, or with negligible error.

2. Introduction

2.1 Other animal analogues used to study decomposition

Taphonomic studies are often designed using animal analogues to estimate the processes that a human cadaver would undergo in specific environmental conditions (1-10). These models are required due to the scarcity of available human cadavers and more importantly because of the ethical and cultural constraints that restrict their use. Decomposition trials use a range of different mammalian cadavers or their skeletal muscle tissue (SMT) as analogues for humans including pigs (3, 6, 7, 11-15), sheep (16-19), dogs (1), rats (5, 20), guinea pigs (2), deer (21), bison (21) and rabbits (8, 22, 23). Larger mammals are preferred for many surface decomposition studies, due to the accelerated rate of decay in smaller analogues and difficulties associated with identifying each stage of decomposition (3). Analogues are used to investigate a range of parameters such as insect succession (1, 3, 4, 24, 25), decomposition chemistry (7, 12-15, 26), microbial activity (6, 16, 17, 27) and general decomposition processes (1, 3, 8).

To date, there has been minimal investigation into the appropriateness of using animals as models for human cadavers in decomposition trials. Two studies have been carried out comparing pig cadavers with human cadavers and comparing pig cadavers of several different sizes with human cadavers and analyzing the insects that colonized the bodies during decomposition (9, 28). Of the insects captured at the pig and human cadavers, 99.67% were common to both species, with only very rare insects being found exclusively at either one. Thus,

there was no apparent preference for either cadaver species based on the entomological and statistical analysis (9, 28).

Reed (1) created an insect succession list based on the decomposition of 43 canine cadavers. These data were compared with insect succession in human cadavers (29). An overlap in the insects of forensic interest associated with both cadaver types was observed. While there is an apparent similarity in the insects colonizing human cadavers and two mammalian sources (porcine and canine), there are little available data for making a comparison with other mammals. In a later study, Mann et al. (30) acknowledged that decomposition trials using dog carcasses (20–40 kg) were not comparable with human cadavers (50–65 kg) as the dogs decomposed more rapidly. The only observed similarity was faster decomposition of head regions in both cadaver species, likely related to the facial orifices that attract increased insect activity. A further limitation with the current literature is that the majority of data are obtained for surface decomposition events (1-5, 8, 9, 28, 29), and very little data are available for buried cadavers (6, 7, 29, 31-33) or cadaver analogues (12-17, 19). This is often related to the exclusive investigation of entomology associated with cadaver decomposition which typically requires surface exposure. Hence, burial environments are markedly under investigated in the sphere of forensic taphonomy.

In a clandestine grave, a decomposing cadaver is in intimate contact with the surrounding environment, commonly soil. Soil is composed of many different components: these include organic material from plants and animals, mineral matter, and a living component of macro,

meso, and micro faunal populations (10, 34, 35). These components are interlinked, and changes to one aspect will generally lead to variations in the others. However, quantification of these variations can be difficult. The decomposer subsystem of the terrestrial environment has two major functions: mineralization of essential elements and formation of soil organic matter (36). A cadaver is a rich source of both essential elements and organic molecules and therefore becomes a hot spot for biotic and abiotic transformations (10, 34, 37).

The introduction of a cadaver to a soil environment, therefore, has an acknowledged impact on the surrounding soil chemistry and microbiology. However, soil physicochemical properties and microbial community change after the burial of cadavers or cadaver analogues from different mammalian species remain poorly described. The purpose of the study carried out by Stokes *et al.* (38) was to investigate the decomposition of human and other mammalian SMT in a soil environment to determine the potential of animal analogues to be used as models for human SMT in decomposition studies. The authors were interested to see whether decomposition rates, microbial activity, and changes to soil chemistry (including nutrient release) differed between mammals whose muscle tissues were easily available for taphonomic research. In their experiment, they chose to compare porcine, ovine, and bovine SMTs with human SMT using established experimental techniques. Measurements were carried out on detritosphere soil and included pH, potassium, phosphate, ammonium, and nitrate. Microbial activity was also monitored over the course of the study.

2.2 The use of pigs as a human analogue in decomposition studies

The origin of pigs as human proxy can primarily be traced to entomological studies where the use of animal proxies for the comparison of insect succession throughout decomposition is well tested. Rodriguez and Bass (29) compared available insect succession data collected from canine cadavers (1) to that observed on human cadavers and found significant overlap. It appears that the majority of insect species (99.67%) show no preference between pigs or humans (9, 28). However, caution is warranted when using entomological studies to validate the use of pig carrion in decomposition studies. While the entomologist and anthropologist both seek to understand PMI, it is incorrect to assume that the disciplines are studying the same underlying physiological phenomenon. The entomologist is concerned with insect development, progression, and succession guided by the sum of the decomposition event (e.g., the release of chemical constituents that guide chemotaxis, moisture content and related patterns of oviposition, and visual and mechanosensory cues that govern insect movement). Conversely, the anthropologist is concerned with the sum of stages of gross morphological change, which may correlate with various stages of decomposition (e.g., rate, chronology, and presentation of tissue dissolution under various circumstances). Therefore, the biological features that make pigs a viable research tool in entomology cannot be assumed to translate to taphonomic investigation.

Early observational studies on decomposition used a variety of species, including cows, seabirds, and sea mammals (39). Payne (3) studied dogs, cats, squirrels, rabbits, chickens, birds, and pigs, concluding that relatively large animals of uniform size were best for his studies (40).

The results of these studies were rarely compatible and consistently resulted in opposing criteria and sequences used to describe the trajectory of decomposition. Micozzi (40) suggested that the incompatibility was due to the wide variety of animals used and the conditions under which the studies were carried out. Stokes *et al.* (38) evaluated potential proxies for use in soil decomposition studies. A suite of physiochemical soil characteristics was compared among and between tissue types using small samples of skeletal muscle tissue from humans, pork, beef, and lamb. Different species exhibited similar patterns of change, but none of the proxies were an ideal predictor of human skeletal muscle tissue for soil decomposition studies. Wang *et al.* (41) conducted a comparative analysis of insect succession on human remains, pig carrion comprising two size categories, and rabbit carrion. They conclude that body size and decomposition rate share an inverse relationship, and insect species diversity and complexity are more complicated on larger remains. Dipteran development rate was consistent across all size and species categories, although a subset of forensically significant Calliphoridae species were unable to complete first-generation development on rabbit carrion.

Dautartis *et al.* (42) observed a similar correlation between body size and trajectory of decomposition. Five each of pigs, humans, and rabbits were used to study gross morphological changes and insect activity across a 5-month interval. The conclusion was that the pattern of decomposition differed between the three groups, and they were not likely to be interchangeable for decomposition research. However, the continuing challenge in using human remains for research study is sample size. While these studies present intriguing patterns, there is still a need for research conducted within human samples, using larger

sample sizes, and in differing environments. Additionally, longitudinal studies are warranted in dry areas where desiccation occurs and bodies take much longer than 5 months to skeletonize. The goal of the research done by Connor *et al.* (43) was to further test whether, and to what degree, pigs provide a useful proxy for human decomposition.

3. Discussion and Conclusion

No single SMT was an ideal predictor of human SMT for soil decomposition studies. Several measurements differed significantly between porcine, bovine, ovine, and human SMTs, including microbial activity, gravesoil chemistry, and nutrient concentrations. Microbial activity in soil exposed to porcine and bovine tissue was higher than in soil exposed to human tissue. The concentrations of the nutrients PO_4^-, K^+, and NH_4^+ in detritosphere soil surrounding nonhuman tissue were often significantly higher than those in soil surrounding human tissue. Nonetheless, the overall patterns of nutrient fluxes and chemical changes in nonhuman SMT followed that of human SMT. Ovine tissue was the most like human tissue in many measured parameters. Therefore, no nonhuman tissue types were precise predictors for human tissues in taphonomic studies, but all offered some approximation in decomposition dynamics.

Research done by Connor *et al.* (43) found that in approximately 60% of the pig specimens, the intestines ruptured through the abdomen during the bloat phase; abdominal rupture did not occur in any of the human specimens. While humans and pigs present anatomical, physiological, and genetic overlap, fundamental differences between the two species exist. While both humans and pigs are monogastric, key differences in the structure and function of the gastrointestinal tract (GIT) may affect the rate, timing, and trajectory of decomposition. On average, the human intestine (large and small) is 7.5 m in length, as opposed to 23 m in the pig. The pig small intestine further presents differences in segment length and branching of mesenteric vessels and attendant anastomoses (which facilitate cellular communication with surrounding tissues). In the human GIT, ileal Peyer's patches (PP) are an aggregate of lymphoid

modules that facilitate an immune response within the mucosa by monitoring intestinal bacteria and inhibiting growth and spread of pathogenic strains. Conversely, the ileal PP in pigs is continuous, which not only constitutes a structural difference, but also implies major differences in typical bacterial load, potential exposure to pathogenic bacteria, and enteric colonization. A pyloric diverticulum—a site for microbial metabolism of ingesta—is present in pigs but absent in typical human anatomy (44). Finally, the cecum, ascending and transverse colon, and the proximal portion of the descending colon are oriented in a series of centrifugal and centripetal coils to accommodate length and orientation associated with quadrupedalism, which differ from the folded and "stacked" structure of the human intestines.

The sum of these many subtle differences may contribute dramatically to gross differences in decomposition, such as the abdominal rupture observed in this sample. In more basic terms, while this phenomenon is likely multifactorial, a significantly longer GIT summarily constitutes larger cubic volume of lumina within which decompositional gasses have the potential to aggregate, resulting in greater potential for eruptive response. Overall similarity in TBS scores in pigs and humans in early decomposition may be an artifact of the overfitting of data to the scoring system; data collection necessitates that a score be applied so the "best choice" category regardless of overall technical accuracy. Only six categories in the head/neck and five in the trunk and limbs describe early decomposition. Limited scoring options may reduce or eliminate the resolution necessary to highlight divergence in gross change. Keogh *et al.* (45) used the Megyesi *et al.* (46) total body score (TBS) to define the typical trajectory of human decomposition and compared this to observations made within a sample of 20 pig carrion. The

authors concluded that the gross changes observed between the two groups were sufficiently different to warrant a separate scoring system for pig carrion. However, TBS comparison was limited to pigs and did not include humans in the sample environment, so the differences observed may be a combination of species and environment.

Compared to the human sample, the pigs were a more superficially homogenous sample at the start of the project; control over variables such as weight, cause of death, reported pathology, and immediacy of placement within the facility reduced intragroup variation. Such a homogenous sample may allow researchers to control variables and identify trends in decomposition, but identified trends need to be validated within human samples before their efficacy can be accurately reported and extreme caution is warranted when attempting to validate a study conducted on humans within a pig sample. The use of pigs in research has the potential to complicate judicial proceedings where researchers are asked to testify on the condition of human remains based on their knowledge of pig decomposition, or where established methods are called into question under the Daubert Standard following publication of research conducted within pig samples.

The human remains were a more variable group, but a more realistic sample of forensic cases. Because forensic cases often involve unknown circumstance, the variation among the human sample was in fact reflective of the variation that any useful decomposition measure must encompass to provide an estimate of the PMI. In this sense, discerning patterns among homogenous proxy populations may provide a false sense that the same conclusions are

applicable to a much less homogenous population. Forensic disciplines are still attempting to understand how the interaction of complex variables affects human decomposition—a conservative approach is critical when drawing direct correlations between humans and proxies. The sum of the anatomical, physiological, and pathological differences presented may be manifest in unexpected ways at any time throughout the decomposition event. As researchers struggle to understand the diverse complexity of human decomposition, it is specious to suggest that the intersection of captivity, dynamic zoonotic disease processes, death, and decomposition may be overlooked and a distinct and diverse species defined as homogenous to meet analytical needs.

Finally, the TBS model used by many decomposition studies does not work well with pigs and, for the data presented, fit neither pig nor human data in late-stage decomposition. This was likely due to the desiccation seen in the Forensic Investigation Research Station's (FIRS) semi-arid environment and highlights the need for region-specific validation of models used to estimate postmortem interval.

When compared using the TBS model, the trajectory of decomposition observed between human and pig samples diverged in rate and gross presentation. Additionally, the TBS scoring model did not perform well in the arid environment at the FIRS. Regional decomposition patterns may require regional scoring models. Nonhuman proxies do provide a superficially homogenous sample allowing isolation of individual variables and have the potential to indicate trends in taphonomy. Human samples tend to be more variable, particularly in body

composition and cause of death, both of which affect the pattern of decomposition. Pigs may be useful in studying general trends. However, they are not a substitute for human subjects, and caution is warranted when attempting to apply data derived from pigs to human subjects, especially in medicolegal investigation. Above all, reliance on a relatively homogenous proxy sample may make researchers overconfident in their ability to predict the timing and patterns of decomposition.

The use of nonhuman animal proxies for human remains in decomposition studies has been based primarily on the assumption that most carrion, especially those that are similar in size and integument, will undergo the same decomposition process at an analogous rate as humans, or with negligible error. However, Moreau *et al.* assert that "Until more peer-reviewed studies are published that directly test the pig-as-surrogate claim, it appears that the use of medium-sized pig carcasses [22–27 kg] relies more on logic and practicality than experimental evidence" (47). Locally available nonhuman animals are fully appropriate for ecological studies designed to investigate arthropod variation within a particular environment or to address questions concerning pest control, soil sciences, wildlife management, forestry, and natural scavenging behavior. The results of the study conducted by Dautartas *et al.* (48) demonstrate, however, that human remains behave less predictably than those of pigs or rabbits such that the nonhuman models could not replicate the impacts of differential insect activity, scavenging or physical state changes (e.g., mummification) exhibited by the human subjects. While this paper seeks to document the decomposition pattern differences among carrion species, subsequent papers will focus on different variables responsible for those pattern variations. Seasonality also

plays a role in differential decomposition as pigs and rabbits decompose faster than humans when insects are present and scavenging was more extensive in the winter. In sum, the study indicated that human data are best for determining human patterns of decomposition in forensic cases and should form the basis for research seeking to develop forensic-related PMI methods.

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