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Determining an accurate method for estimating the post-mortem interval of decomposed remains found in a temperate Australian environment

Presented by

Stephanie Jane Marhoff-Beard

Primary Supervisor

Dr Hayley Green

Co-supervisor

Professor Shari Forbes

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Statement of Authentication

The work presented in this thesis is, to the best of my knowledge and belief, original except where acknowledged in the text. I hereby declare that I have not submitted this material, either in full or in part, for a degree at this or any other institution.

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Stephanie Jane Marhoff-Beard

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List of Abbreviations

ACT   (Australian Capital Territory)

ADD   (Accumulated degree days)

BoM   (Bureau of Meteorology)

H_2S (Hydrogen Sulphide)

PMI   (Post-Mortem Interval)

r^2 (r squared value)

RAAF (Royal Australian Air Force)

SPSS   (Statistical Program for Social Sciences)

TBS   (Total Body Score)

Abstract

Estimating the post-mortem interval (PMI) is one of the most important determinations to make in a forensic investigation. However, at present, an accurate and reliable forensic anthropological method for estimating the PMI, based on the gross morphological changes occurring during decomposition, is currently unavailable. This is due to a multitude of variables influencing the rate and processes of decomposition in any given environment. Forensic anthropologists have traditionally relied on their knowledge and experience of the decomposition stages to make an assessment of the time since death. However, recently new, quantitative methods that are not solely based on the anthropologists observations, have been developed in a number of regions that have been proposed to accurately determine the PMI based on the observed decomposition changes alongside important taphonomic variables.

The aim of the current study was to examine and document the decomposition process of pig carcasses, as an analogue for human remains, in the summer and winter climate of the Greater Western Sydney region. Secondly, the study aimed to evaluate the accuracy and replicability of the Megyesi et al. [1] ADD method, the Marhoff et al. [2] formula and the Vass [3] universal PMI formula, for their applicability as PMI methods in this region. Thirdly, should the methods mentioned above fail to accurately determine the PMI of remains within this region, a new method for PMI determinations will be created based on the observed decomposition changes and the most influential taphonomic variables affecting decay rates within the Greater Western Sydney region.

Over an 18 month period, from June 2014 to March 2016, four experimental trials were undertaken: two summer trials and two winter trials. Eight adult pig carcasses per trial were left to decompose naturally on a soil surface at Western Sydney University's Hawkesbury campus. During each trial, four carcasses were left to decompose in the shade under the canopy of trees and the other four carcasses were deposited in the open, with direct exposure to the sun. This was to examine the differences in decay rates between a sun and shaded microclimate. The published methods [1-3] and their associated scoring protocols were applied to determine the PMI of the remains.

Through linear mixed modelling, the variation between the true PMI and the estimated PMI. The results showed that of the three methods validated in the present study, none could accurately determine the PMI in the Greater Western Sydney region. The Vass [3] formula overestimated the PMI during the winter trials but underestimated the PMI of the summer remains. The Megyesi et al. [1] and Marhoff et al. [2] methods were both found to underestimate the PMI when they were applied during the winter but overestimated the PMI when they were applied during the summer.

As it was found that the currently published protocols for PMI estimates could not accurately determine the PMI of remains found within this region, a new method (the Marhoff-Beard method) for PMI determinations specific to the Western Sydney region was created. Using the degree of soft tissue decomposition observed at the time of discovery alongside the climatic variables humidity, wind speed, and rainfall, new regression equations were created.

To determine if the new Marhoff-Beard formulae were accurately estimating the PMI for the Western Sydney region, the method was validated retrospectively from photographs of pig and human remains, and was applied longitudinally from the start to the end of the decomposition process on a donated human body. The validation showed this new method can accurately determine the PMI in a Western Sydney winter and summer climate and results were comparable when it was applied to both human remains and pig carcasses. The method performed consistently well during the fresh and early decomposition stages with a maximum error of eight days. As the remains dried and progressed through the advanced and skeletonisation stages, the accuracy of the method became compromised. It is likely the Marhoff-Beard method failed after this time point, as the decomposition process during the later stages is affected by further variables which were not accounted for by this method.

Continued testing of the Marhoff-Beard method for PMI determinations should be undertaken both within this region and other temperate Australian locations. It should also be determined what variables are affecting decay rates during the more advanced stages of decomposition as this will help refine the PMI formula for its use during these stages.

Chapter One

1 Introduction

The discovery of human remains leads to two important questions, the first, who is the individual? Secondly, when did this person die? If the remains discovered are fresh or relatively fresh it is the role of the forensic pathologist to extrapolate answers from the condition of the body to answer these questions [4]. However, if the remains are in a greater state of decomposition or skeletonised, an investigation in to whether the remains are of forensic (anthropological) or archaeological significance is first conducted [5, 6]. If it is determined the remains are of anthropological significance, the forensic anthropologist will make the determinations necessary for identifying the individual, ascertaining the manner of death and determine the time since death [6].

While the estimation of time since death, commonly referred to as the post-mortem interval (PMI), is of critical importance in a forensic investigation, it is one of the most difficult determinations to make [5]. The longer the time elapsed since death, the more imprecise are the chronological or sequential indicators that remains present throughout the decomposition process and, subsequently, the broader the estimated time period [7, 8]. This is due to the great number of variables interacting with the body, altering the rate of the decomposition changes it undergoes [4, 8].

Despite the impact of external and internal variables influencing decomposition, decomposed remains can provide forensic anthropologists with crucial information as to the time transpired since death [4, 7]. An accurate determination of time since death can provide police with a timeline, ultimately, focusing the direction of the forensic investigation. It can also assist police in homicide cases where remains can corroborate witness testimony and exclude potential suspects by supporting the suspects alibi [6].

A multitude of decomposition studies that are concerned with accurate PMI estimates have been undertaken, yet they have largely been performed from an entomological perspective which looks at the presence of insects on a decomposed body [9]. This was until recently when forensic anthropologists began researching and highlighting

the other taphonomic variables influencing decay rates [9]. Entomological evidence is not always found at each of the decay stages and, as such, it is important to understand how other factors affect the prevailing changes remains undergo throughout decomposition. This has provided forensic professionals, such as anthropologists, with a more holistic approach to investigating human remains and allows for a more accurate estimation of time since death to be made when a variety of variables are considered.

Climatic variables, such as temperature, have been found to have one of the most significant effects on decomposition rates, influencing both the rate and processes of decay [9]. This knowledge has prompted many taphonomic and anthropological studies on the effects of temperature on decomposition, predominantly in the United States [1, 9-12] and Canada [13, 14]. As a result of these studies, researchers have concluded that decomposition is a climatic and geographically specific process because no two studies presented the same results regarding the sequence and rate of decay [9, 11, 12, 15]. As such, the results obtained in these studies may not be applicable elsewhere due to the differences in microclimates around the world [12, 16-18]. Given the geographically specific nature of decomposition and that every region worldwide possesses its own unique climate and environment, further studies need to be undertaken to understand and examine how these variables affect the rate of soft tissue decay in other individual regions [7].

Information on how a temperate Australian environment affects the decomposition process of remains is very limited, with few studies investigating the effect this climate has on both soft tissue decomposition and the degradation of bones [2, 19, 20]. At present a reliable method for estimating the PMI of decomposed remains in an Australian context that makes use of the soft tissue changes occurring to remains during decomposition is currently unavailable. Decomposition rates of human remains are climate dependant, thereby, making current published methods for estimating PMI not applicable in Australian environments. Thus, Australian specific standards need to be developed. As the soft tissue changes occurring to remains in decomposition are significantly correlated to climatic variables, it is these factors that can be used to accurately determine the PMI within a number of days.

It is well recognised the need to develop accurate methods for estimating PMI is of increasing necessity. However, despite research into developing methods that

exclusively address the extended PMI's that are the purview of forensic anthropologists, limited accuracy has been achieved [4, 7, 8]. Current anthropological methods are qualitative and require the anthropologist to rely on their understanding of the decomposition stages and the relative time it would take to achieve the observed state of decay [12, 16, 21]. While these decomposition stages have been thoroughly researched, and documented, their use as the sole method of estimating PMI is unreliable as each forensic anthropologist has their own interpretation of these stages. Qualitative descriptions such as these establish wide time parameters for when death occurs that can differ from the actual date of death from days to weeks to months, as they are not time regulated. These variations have, thus far, impeded the development of an appropriate standard method for estimating PMI based on the degree of soft tissue decomposition.

Furthermore, qualitative methods rely on the experience and skill of the forensic anthropologist and they are difficult to justify in a legal setting under standards applying to expert witness testimony and evidence (NSW Evidence Act 1995) [22]. While considered to be expert knowledge, they are still considered opinion and are, therefore, subject to bias. The NSW Evidence Act 1995 Section 79 Subsection 1 states: *"If a person has specialised knowledge based on the persons training, study or experience, the opinion rule does not apply to evidence of an opinion of that person that is wholly or substantially based on that knowledge"* [23]. Quantitative methodologies are based on numerical data and are considered less subject to bias as they have the potential to be reproduced and will be more acceptable as expert evidence.

With these research gaps in mind, the current study will have the following contribution to the field of forensic anthropology. Decomposition studies, to date, have predominantly been conducted in Canada [13] and the United States [24], with other studies being undertaken in areas of Europe [25] and South Africa [22]. The current project will explore how the methods for PMI estimates from international studies can be applied in a different climatic setting. While the pilot study in 2013 [26] suggested that current international methods are not suited for local climates, negative results in that study may be attributed to the limited sample size and experimental period. The present study will act as a validation of the results of the pilot study [26]. The development of a new quantitative method for estimating the

PMI of decomposed remains specific to an Australian context will increase the accuracy of PMI estimations from weeks and months to days, making it a method that can be applied in actual forensic cases.

1.1   Taphonomy and Forensic Anthropology

Taphonomy is defined as the discipline or study of the environmental conditions and processes which affect the preservation of an organism after death [27, 28]. What was originally a branch of palaeontology, taphonomy has been adopted by forensic anthropologists, and many other forensic disciplines such as entomology and botany, to understand the relationship between the decomposed human body and how the morphological changes which occur to the body in death, are influenced by the immediate environmental region the body is located in and the passage of time [29, 30].

Taphonomic research relies on observations of the gross anatomy of decomposing remains to gather qualitative or descriptive data for the purpose of developing baseline data to aid in the identification of decomposition processes and sequences in future forensic investigations. Knowledge of the characteristic changes a body can or will undergo after death and how the environmental variables affect these changes, plays a pivotal role in the calculation of the PMI [8].

Over the past sixty years, forensic anthropological research has increased considerably as more researchers discovered the benefits of taphonomy in forensic cases [31]. During this time, a multitude of studies have been undertaken to examine the effect of the numerous environmental variables on a body and have utilised a number of different analogues where human remains have not been available. Examples of the models used comprise of dogs [21, 32], rabbits [33-35], sheep [36] and pigs [20, 37, 38], as well as human bodies [16, 39, 40] where possible. These studies include investigations into the effect of hot and cold climates [41, 42], water submersion [43, 44], the effect of a variety of soil types [45], clothed and unclothed remains [46], burned bodies [47], effect of burial type [48] and more. Each of these variables influences the decomposition process in a different way, but will ultimately either accelerate or retard the rate of decay.

Knowledge of how these variables influence the process of decay aids in distinguishing peri-mortem, ante-mortem and post-mortem injury and can narrow the

window for time since death determinations by forensic anthropologists. It is widely accepted that decomposition sequences are highly geographically specific as each region has its own unique climate and environment [12, 16, 18, 49-51]. Decomposition sequences have been documented for a number of regions worldwide including regions of the United States such as Texas [52], Montana [53] and Tennessee [12], regions of Canada, such as Alberta [54] and Nova Scotia [14], Spain [49] and South Africa [55], however, few regions within Australia have been thoroughly investigated. In order to gain a better understanding of how the unique environmental features of temperate Australia affect the decomposition process, further taphonomic and anthropological studies must be undertaken if PMI determinations are to be narrowed.

1.2   The stages of decomposition

The control of living cells by metabolic pathways is essential for sustaining life and maintaining the integrity of the human body [56]. However, after death the control of these cells by these metabolic pathways is lost, leading to a succession of chaotic events that result in the body undergoing decomposition. As a body undergoes decomposition, an extensive number of complex changes occur and it is these changes in the gross anatomy that have been categorised by researchers and anthropologists into a number of broad stages of decomposition.

Over the past sixty years, extensive research has been undertaken to better understand the changes occurring in decomposition and to better refine the stages of decay for application of time since death estimates [1, 12, 16, 20, 21, 47]. One of the first taphonomic studies that identified the decomposition sequence and which was characterised into a series of stages was conducted by Reed [21] in 1958. The purpose of the study was to investigate the role of carrion insects in the decomposition process of canine remains. Reed [21] reported that decomposition occurred in four broad stages: fresh, bloat, decay and dry, and he concluded that although these stages occur sequentially there are further factors such as insect activity, vertebrate scavenging and environmental factors which influenced the decay process. He suggested that due to these influential factors, the rates of decay he described and observed in his study may not occur at the same rate elsewhere [21].

Exploring Reed's [21] study further, Galloway et al. [16] investigated the effects of an arid environment on remains in the Arizona-Sonoran desert in 1989. This retrospective study was performed using local forensic case files to evaluate the effects of this specific desert environment and how it influenced the decay process of human remains. Galloway et al. [16] described the decomposition process in five stages: fresh, early decomposition, advanced decomposition, skeletonisation and extreme decomposition, as opposed to the four broad stages identified by Reed [21]. Galloway et al. [16] drew similar conclusions to Reed [21], in that while they reported certain characteristic changes in this microclimate, most notably mummification, this would not be observed in all locations, suggesting different microclimates would produce their own unique effect on remains. The following descriptions of the stages of decomposition and the changes occurring within these stages, will follow those defined by Galloway et al. [16].

1.2.1 The fresh stage

Galloway et al. [16] classify a body as fresh when gross soft tissue decomposition and insect activity has not yet commenced. The precursor to gross decomposition first occurs at a cellular level within the body, a process known as autolysis, where the self-digestion of cells via an enzymatic reaction takes place. Visually, this may first be observed at the junction of the epidermis and dermis, where the cells begin to lose their structural integrity resulting in skin slippage [57]. It is in this stage that the 'classic triad' of decay, livor mortis, rigor mortis and algor mortis, occurs.

1.2.1.1 Livor mortis

Livor mortis, also referred to as lividity, is one of the earliest changes observed in death. While the individual is alive the heart is functioning, circulating blood around the body, however, when death occurs, circulation ceases and due to gravity the once circulating blood begins to settle to the lowest portion of the body [13, 58]. For example, if a body is lying in the supine position, blood will settle along the individuals back and along the posterior of the arms and legs.

A purple-red discolouration is observed on the body where the blood has settled. The colour of lividity is variable and changes from dark pink to red to purple overtime [58, 59]. This is due to oxygen disassociating from haemoglobin resulting in deoxyhaemoglobin, subsequently forming the purple pigment [58-60].

1.2.1.2 Rigor mortis

Rigor mortis is the extended contraction of the body's muscles causing it to become rigid. As the sarcoplasmic reticulum of muscle cells deteriorates it releases calcium into the cytosol where an influx of calcium ions is admitted to the sarcomeres [56]. Here calcium activates the Myosin-Actin Cross Bridge by unblocking the binding site on the actin protein filament allowing myosin to bind instead [56]. The Cross-Bridge retracts and the sarcomeres become joined, shortening the muscle length and causing muscle contraction [59]. In life, ATP-dependant pumps propel calcium out of the cell and back to the sarcoplasmic reticulum to release the Cross-Bridge and induce muscle relaxation, however, ATP is not produced in death, thus, the state of contraction persists until the muscle cells start to decompose [13, 59].

Rigor mortis begins in the muscles of the eyes and face as they are smaller than the other muscles and it continues to spread throughout the muscles of the body from the neck, trunk, upper limbs and then finally the lower limbs [22, 58, 59, 61]. Goff [58] noted two factors in particular affect onset and duration of rigor, they being temperature and metabolic state of body. Lower ambient temperature accelerates onset and prolongs the duration of rigor while warmer temperatures delay rigor. If at the time of death fever is present or vigorous physical activity exerted, rigor may present earlier than usual due to the increased levels of lactic acid produced when muscles are active [59].

1.2.1.3 Algor mortis

Algor mortis is the cooling of the body. In death the body ceases to regulate its internal temperature due to the cessation of homeostasis, thus, the internal temperature of the body begins to align with the ambient air temperature by actions of convection, radiation, conduction and evaporation [58-60].

Myburgh [22] identified three distinct periods in algor mortis. Initially, a temperature plateau occurs representing the first three hours since death. Secondly, the intermediate period when rapid cooling occurs. And thirdly, the terminal or end phase when the rate of cooling has reached equilibrium with the ambient air temperature [22].

Chemical reactions within the body occur at approximately 37°C but as the body cools, the rate of metabolism affecting enzymatic activity declines due to a loss in the

production of ATP as a fuel source for metabolism [22]. This results in an inability of cells to carry out their role in repair, regeneration and biosynthesis and in doing so makes way for the procession of the putrefactive stages of decomposition (early-advanced decomposition stages) [58, 61].

1.2.2 Early decomposition stage

Putrefaction gives rise to the most dramatic and obvious soft tissue changes in decomposition. As the cells of the deceased individual reach end-stage autolysis, an almost entirely anaerobic environment exists promoting the rapid proliferation of endogenous anaerobic bacteria inhabiting the gastrointestinal tract such as *anaerobic lactobacilli, anaerobic streptococci* and *clostridia* [13, 22, 59]. Microorganisms implicated in the putrefactive process are concerned with the degradation of carbohydrates, proteins and fats for their own nutritional supply and growth resulting in the accumulation of gases and acids responsible for the characteristic colour changes, bloating and odour of decomposed remains [13, 59, 61].

Galloway et al. [16] describe the appearance of a green discolouration of the skin is the earliest sign of the commencement of the early decomposition stage and the putrefactive changes. Beginning at the right iliac fossa above the region of the caecum (the first part of the large intestine) where the contents of the bowel are more liquefied and bacteria levels high, this discolouration then typically spreads to the anterior abdominal wall, trunk, neck, face and finally limbs [22, 59, 60]. This occurrence is caused by the breakdown of protein products such as amino acids by bacterial enzymes to generate hydrogen sulphide gas (H_2S) [59, 61]. H_2S can readily diffuse through tissue and when it comes into contact with blood products it reacts with haemoglobin to produce sulf-haemoglobin; a green pigment [13, 61].

The green discolouration will typically develop over a period of days by which time the entire body can take on a greenish hue with a red-purple or green-black 'marbling' effect where the sulf-haemoglobin has invaded the blood vessels giving them a more pronounced effect under the skin [61]. As decomposition continues, H_2S reacts with iron forming ferrous sulphide; a black precipitate and, as such, the colour of the body will change in intensity from green to black.

In this stage, proteins and carbohydrates are broken down leading to the production of gases such as hydrogen sulphide, methane, ammonia, putrescine, cadaverine and

carbon dioxide [61]. It is these gases which are responsible for the malodour generated during decomposition [59, 61]. The gases generated accumulate in the abdomen and give rise to 'bloating'; a phase whereby the build-up of gases continues, causing distension of the abdomen [62].

Other important changes observed during decomposition include swelling of the face from gases, skin blisters and purging of decomposition fluids through the mouth, nose and anus [22, 58]. Due to the rise in internal pressure as a result of gas accumulation, protrusion of the tongue and eyes can be observed as well as eversion of the lips [22, 58]

It is during this stage and the processes that transpire where significant insect activity in the form of flies and maggots emerges. According to Simmons et al. [15], maggots account for the largest amount of destruction to the soft tissue of a body by a single organism. Not only do they consume the flesh but also take refuge within the body cavity so as to continue consumption and reproduce.

1.2.3   Advanced decomposition stage

After boating subsides, advanced decomposition commences. During this stage, significant amounts of soft tissue have been degraded by microbial and insect activity, resulting in the exposure of the underlying skeletal structure in some areas. As internal organs liquefy from bacterial destruction and are purged from the various bodily orifices, the abdomen and neck collapse and the tissues sag [62]. The soft tissues of remains begin to dry and desiccate and can begin to mummify if the environmental conditions are optimal [16]. This stage also marks the end of the putrefactive changes of decay.

1.2.4   Skeletonisation stage

Skeletonisation is considered complete if soft tissue is no longer present and insect activity has ceased [58, 63]. It is not uncommon for the attachment points of ligaments to remain attached once all other soft tissue has been removed, generally at the articular ends of long bones and along the vertebral column. Bones may retain a greasy exterior and remain discoloured until the drying process is complete [16].

1.2.5   Extreme decomposition stage

End stage skeletonisation shows the bones to be smooth and clean. For remains discovered in an exposed or unprotected environment such as full exposure to the sun and the elements, a series of changes can occur transforming the appearance of the bone. In the first six months since skeletonisation occurred, bleaching will be apparent whereby the bones dry out and lighten in colour from sun exposure [61]. This destruction to the bones occurs over a lengthy period of time and is referred to as the extreme decomposition stage; the fifth and final stage of the decay process defined by Galloway et al. [16]. During the decay process of bone, protein or bone mineral is lost leaving the bone weak and brittle resulting in the breakages, cracking, exfoliation, flaking and warping that is observed during the decomposition of the bones [61].

In 1978, Behrensmeyer [64] recognised six progressive stages of bone weathering in mammals for a typical Kenyan environment to assist in the understanding and reconstruction of PMI for older remains. Although forensic cases generally observe a much shorter PMI, knowledge of the natural changes to bone can assist in identifying what is natural and what is peri-mortem trauma [64].

As mentioned previously, the stages of decomposition discussed above follow a relatively sequential order, however, there is no obvious demarcation when one stage ends and the next commences [65]. It is important to understand that there is no precise or exact timeframe for when each stage presents during the PMI. The rate at which remains enter the various decomposition stages will differ amongst remains as the rate of decay is highly influenced by the taphonomic variables of the immediate environment [66]. Yet knowledge of the stages and the changes of decomposition is necessary for estimating the PMI of a deceased individual. The present study aims to examine the decomposition process of remains in the Greater Western Sydney region of temperate Australia during the summer and winter seasons to provide baseline data that may be used in future death investigations when remains are discovered.

1.3   Alternate states of decomposition

A multitude of factors (e.g. temperature, burial) affect the rate of decomposition and although the gross morphological changes a body undergoes in decomposition is relatively consistent amongst remains, there is some variability in these changes [22].

Modifications to decomposition such as mummification or the formation of adipocere may arise as a result of environmental effects [22]. These alternate states of decomposition are discussed below.

1.3.1 Adipocere

Adipocere is the transformation of adipose tissue into a yellow-white, wax like substance due to the hydrolysis of fat with a release of fatty acids [13, 22, 60]. The timing of the formation of adipocere is also quite variable where some researchers have observed it to occur within weeks of death [67], others suggest they have found it to develop months after death [45] but complete adipocere formation of the whole body may take years [68]. The formation of adipocere also has the potential to preserve remains by encasing the body, or regions of the body where it has developed, subsequently inhibiting the decomposition process [69].

The immediate microclimate of the remains greatly influences its formation [70]. A warm moist environment, anaerobic conditions experienced with burials and a mildly alkaline pH level is idyllic for the formation of adipocere, however, it can form in a range of environments including on remains that have been submerged in water [70]. Adipocere commonly forms on the remains of individuals who possess a high body fat content overall and will typically first develop in the subcutaneous fats of the cheeks, breasts and gluteal region [60, 70]. As time passes, adipocere becomes paler in colour and hardens to become a chalky, brittle substance [60].

1.3.2 Mummification

Mummification is the dehydration of tissues (desiccation) and occurs under conditions opposite to that which are idyllic for the formation of adipocere [71]. Skin becomes leathery and parchment-like due to dry climatic conditions. Optimal conditions for mummification to occur require a body to be in a dry environment that is either very hot or extremely cold as this not only dehydrates tissues preserving the body, but also inhibits bacterial action and insect activity, retarding the decomposition process [16, 58, 60]. Mummification in temperate climates is uncommon but not abnormal, where it has been observed in remains that have been exposed to forced hot-air heating such as in buildings [60, 63]. In ideal conditions, mummification can be observed in weeks.

1.4   Factors affecting decomposition

The decomposition process of a body is not a time regulated process and the rate at which a body decomposes is influenced by a myriad of both intrinsic and extrinsic variables. Many taphonomic studies have been undertaken to investigate and identify the variables that most affect the decomposition rate and process of a body in a number of geographic regions. The below section presents some of the most influential taphonomic variables that have been suggested to greatly effect decay rates.

1.4.1   Climatic factors
1.4.1.1   Temperature

It has been suggested in the literature that ambient temperature is one of the most influential factors affecting the decomposition process of remains. Warmer temperatures are said to increase decay rates, whereas colder temperatures will retard the rate of decay [9]. It has also been postulated that warmer conditions promote bacterial activity which subsequently accelerates decomposition [72]. However, while exceptionally high temperatures will hasten the rate of decay, they will also inhibit the growth of bacteria [73].

In arid, desert like environments or similar circumstances, high temperatures dehydrate the skin resulting in a hard, leathery outer shell of the body. The mummified skin protects the external body from further decomposition, while internally the viscera continue to decompose [9]. It should be noted that mummification can also occur under very cold but very dry conditions [22].

Cooler climates inhibit the rate of decay and in some circumstances, will prevent remains from decomposing further. Cold temperatures also affect the insect succession. Under cold temperatures, fly eggs and larvae will die if they are exposed to cold, ambient temperatures and are unable to bury themselves beneath the warmth of the body [9].

1.4.1.2   Humidity

The degree of moisture within an environment can greatly affect the rate of decay. Low humidity levels have been found to aid in the desiccation (drying out) and

mummification of remains. Once remains desiccate or mummify, fewer insects are attracted to the remains, thus, preventing further destruction to the body.

Extremely humid environments can accelerate the rate of decay as it can promote insect activity and viability as the larvae require some degree of moisture for continued survival [65]. Prieto et al. [74] reported that remains found near the coastal regions of Spain, demonstrated greater levels of decomposition than remains found in other regions and proposed this was likely due to the climate of the coastal regions which is typically characterised by warm temperatures and high humidity levels; which are the optimal conditions for the proliferation of microorganisms [22].

1.4.1.3　Rainfall

Little research has been undertaken regarding the effect rainfall has on the decomposition process and the studies observing the effect of rainfall all present conflicting results as to its influence. Archer [75] suggests rainfall may increase decay rates as it provides moisture for the proliferation of microorganisms. The combination of rainfall and soil moisture around and beneath a body may slow decay rates by reducing temperatures in the immediate vicinity of the body through evaporative cooling [75]. However, Archer [75] also states, that moist soil around a body will prevent it from drying, thus, providing optimal conditions for maggot survival and bacterial growth which will then continue to break down the body. Heavy rainfall has also been suggested to increase the abundance of insects, however the researchers of this particular study acknowledged that rainfall in combination with higher temperatures and humidity levels may have increased the insect presence on the remains [76]. Rainfall has also been found to rehydrate remains and reactivate the decomposition process [77].

1.4.2　Insect activity

Insect activity is regarded as one of the most influential factors affecting decomposition rates as the presence of insects such as flies, ants and beetles will hasten the rate of decay [65]. While the maggots themselves will consume the flesh of a body, they will also further distribute microorganisms in the body as they move about the remains which in turn accelerates the rate of decay. Large maggot masses can produce widespread damage to a body within a very short period of time and with that activity, produce excess heat furthering the decomposition of soft tissues.

Insect activity, succession and viability are also indirectly affected by other taphonomic variables such as temperature and humidity. Warmer temperatures can increase the number of insects on a body which will ultimately cause greater soft tissue decay. Extremely high temperatures or cold conditions can inhibit their attraction to remains and can result in the death of some of the maggots [65]. If the access to a set of remains is limited, for example if a body located in a room where flies are unable to access the room, this will impede the decomposition process.

1.4.3 Scavengers

Scavengers, such as foxes, dogs, rats, carrion birds (ravens and eagles), aquatic scavengers including sharks and crustaceans (if the body is submerged), will consume the soft tissues and scatter the bones of a body. Mann et al. [9] notes that some scavengers, such as dogs, may consume the bones particularly at the spongy ends of long bones and the pelvic bones.

Regions of the body where animals have consumed the flesh or disarticulated the joints can increase the rate of decay by providing additional access points for insects, accelerate the rate of bone weathering where bones are now exposed, and the disarticulated regions of the body will decompose much faster as they are now smaller and are decomposing independently to the whole body [22].

1.4.4 Body composition

Few studies have investigated the effect of composition, size and weight of a human body on the decomposition process, however, those studies which been undertaken present conflicting results. Mann et al. [9] and Campobasso et al. [65] observed decomposition to occur much slower in newborn babies whereas obese bodies decomposed rapidly likely due to the liquefaction of body fats. Pope's [78] study on the correlation between individual characteristics such as height, weight, body mass index (BMI) and age with the PMI, showed that the weight of the individual during life was significantly correlated with the length of time the body took to decompose. However, Simmons et al. [15], Sutherland et al. [55] and Archer [75] observed small pig carcasses to decompose quicker than larger carcasses. Simmons et al. [15] suggested the slower decay rates of the large carcasses was attributable to the substantial body mass that needed to be consumed by insects.

1.4.5   Trauma

Some researchers suggest that trauma to a region of the body will result in the area decomposing faster than a non-trauma affected site [16, 79]. This is due to the trauma site providing additional easy access points to the body for insects to lay their eggs and feed [16]. Haglund [79] noted that scavenging occurs earlier when trauma is present on a body as vertebrate scavengers are attracted to the wound site and decomposition progresses much more quickly. Cross and Simmons [80] argue however, that there is no difference in decay rates when comparing remains with and without trauma as flies prefer the confines of body orifices but state that injury sites do provide additional points of access for maggots.

1.4.6   Method of deposition (Surface vs. Buried vs. Submersion)
1.4.6.1   Surface

Bodies deposited on the grounds surface typically decompose much quicker than the buried or submerged remains as they are subject to numerous taphonomic variables with little protection from them. For instance, they are exposed to higher ambient temperatures which results in more rapid rates of decay as well as fluctuating humidity levels and rainfall which has also been found to influence decay rates [41].

Remains placed on the surface are more prone to large vertebrate scavengers and insects due to easy access. The microclimate a body is positioned in can also affect the rate of decay. A sun exposed body can decay faster than a shaded body due to the increased levels of solar radiation [13]. The direct exposure to the sun will greatly affect the decomposition and weathering of bone. Bleaching is the lightening of the bones due to exposure of the sun and the heat will also hasten the cracking and brittleness of the bones [16]. The direct exposure of bones to the sun will increase the rate of bone weathering in a shorter period of time, potentially confounding PMI estimates as the bones may appear older than they are [22]. Whereas shaded remains, such as under the canopy of trees, may be better protected from the elements such as rainfall and humidity which keep temperatures cooler, subsequently, slowing the rate of decay.

1.4.6.2   Buried

It has been found that the properties of soil, including oxygen content, degree of moisture, pH levels and temperature of the soil a body is buried in, can influence the

decomposition process [81]. An integral component driving the putrefactive or active decomposition stages is aerobic microbial growth. For this to take place oxygen is required. If oxygen is restricted such as in a burial, microbial proliferation is unable to advance resulting in much slower rates of decay [13]. Soil properties, such as those mentioned above, can also promote the formation of adipocere if the conditions are optimal (e.g. low pH, calcium and microbial activity, high phosphorous levels) which has been proposed to have a preservative function [70, 82]. The deeper a body is interred in a grave, the better protection it has from the taphonomic factors at the surface which includes high temperatures, solar radiation and scavenging. Temperatures are also generally cooler and more stable at a deeper burial level, preserving the body [22].

1.4.6.3 Submersion

As with surface depositions and burials, the decomposition of submerged remains relies on the immediate aquatic environment. Cold or freezing waters will delay the progression of decomposition when compared to warmer waters and the deeper a body is submerged, the cooler water temperatures are, subsequently impeding decomposition [83, 84]. Remains submerged in a body of salt water will decompose slower than when in fresh water, as the salt concentration inhibits microbial growth [84]. The decomposition of remains in freshwater may also be accelerated if the bacterial content is high [85]. Aquatic environments also have their own unique range of scavengers, including sharks, fish and crabs, which will both consume the flesh and disarticulate the body [85].

1.4.7 Clothing and Coverings

Clothing and coverings (e.g. blankets, wraps and fabrics) can both directly and indirectly affect the decomposition process and rate of a body. The type of covering a body is shrouded in has been suggested to have an effect of the decomposition process. Dautartas [72] found that bodies covered in a cotton blanket mummified but those bodies wrapped in plastic tarps prolonged the moist decomposition stage and insects remained for longer. Clothing has been reported to protect the body from direct sunlight which maggots avoid [9]. They instead burrow beneath the clothing to take shelter and feed and will typically migrate to areas where there is some looseness in the clothing such as at the armpit or collar [9]. It has also been

postulated, that tighter or heavier clothing can delay post-mortem cooling, which in turn can hasten the rate of decay through microbial proliferation [22].

1.5 Post-Mortem Interval

The PMI, also referred to as the time since death, is the time that has transpired from the time of death until the date the remains are discovered. An accurate estimation of the PMI is vital for police and forensic investigations as it can provide police with a timeline, ultimately focusing the direction of the investigation [86]. Decomposed human remains can deliver forensic anthropologists a plethora of information about not only the time since death, but also the identification of an individual, the manner of death and the ability to distinguish environmental effects on remains from peri-mortem injury. Remains can also assist in homicide cases by corroborating witness testimony and excluding potential suspects by supporting the accused's alibi.

Despite the importance of PMI estimates, it is one of the most difficult calculations to make due to the vast number of variables influencing decomposition rates, thus, complicating an accurate determination. Many researchers have investigated the decomposition process in an attempt to demarcate where each decomposition stage begins and ends in the PMI and how long a body typically remains in each stage with consideration to the main taphonomic variables affecting the decomposition process. While the stages of decomposition are highly influenced by both intrinsic and extrinsic factors, the gross morphological changes occurring to a body appear in a relatively ordered sequence and, as such, are still useful in PMI determinations.

The development of new and accurate quantitative methods for PMI determinations is of increasing importance. There are currently a number of methods and techniques available from a diverse range of forensic disciplines which are used or have been proposed to accurately determine the PMI, all of which have varying levels of accuracy and can estimate the PMI to within months, weeks or days of the true PMI depending on the method [87]. The greater the accuracy of a method, the longer and more complex the laboratory analysis is, thereby delaying the start of a forensic investigation [3]. The following section presents some of the methods and techniques currently employed to estimate the PMI where they are used for short range, mid-range or long range PMI estimates [66].

1.6   Current methods for PMI estimations

1.6.1   Short range estimates (minutes, hours)

Within the first 72 hours of death establishing the PMI is typically the responsibility of the forensic pathologist. Early decomposition changes such algor, livor and rigor mortis are often used by the forensic pathologist to estimate the PMI with reasonable accuracy during this time period [88]. While there is some predictability as to when the 'classic triad' occurs, there is some variability in the appearance of these changes, particularly algor and rigor mortis, as they a heavily influenced by ambient temperature [66]. More analytical techniques investigating the potassium and hypoxanthine content in the vitreous humour [89], degradation of blood products and cerebrospinal fluid may also be used during the early decomposition period to estimate the time since death [90, 91].

1.6.2   Mid-range estimates (days, weeks)

Over the past 150 years, forensic entomology has gained considerable attention in the literature with regards to the attraction of insect species to a body and their relation to the PMI [86]. Buchan and Anderson [86] suggest that due to the substantial amounts of research undertaken in this discipline, forensic entomology is the most accurate method currently in use.

Estimating the time since death using entomological evidence is based on the concept that the rate of development of an insect species is related to ambient temperature, referred to as accumulated degree days (ADD) [92]. ADD are the number of heat energy units (thermal energy) required for biological and biochemical reactions to take place in a body. As remains progress through each of the decomposition stages, a new influx of insect species is attracted to the carcass and will begin to reproduce. Once the different species attracted to a body at the various stages of decay are known for a specific environmental and geographic region, the use of entomological evidence can be applied to estimate the PMI.

In their 1983 study, Rodriguez and Bass [12] analysed the pattern of insect succession to remains and attempted to link the pattern with the observed decomposition stages in an attempt to estimate the time since death. They concluded that due to the sequential nature of both insect succession and the decomposition

changes, an estimate of PMI could be generated when employing entomological evidence.

Researchers caution however, there is some variability associated with the succession and development of insects [93]. O'Flynn [51] found in their Australian study that blowfly populations differed between the various regions of Australia because of the differences in the climate in Western, Eastern, Northern and Southern Australia, suggesting that the results obtained in one region are not applicable to another; they are geographically specific. King [93] notes that exceptionally warm temperatures or cold temperatures will affect the rate of development of larvae and Anderson and VanLaerhoven [94] postulate that rainfall can limit or prevent oviposition. It has also been found that insect activity may not be present in the more advanced decomposition stages, making it difficult to estimate the PMI when insects are no longer present [88].

Given the variability of insects, it may be more appropriate to apply a PMI method that utilises the decomposed body itself; a role that is required of a forensic anthropologist.

1.6.3   Long range estimates (weeks, months, years)

Typically, anthropological methods for estimating the PMI rely on the experience of a forensic anthropologist and their knowledge of the decomposition changes and the relative time it takes to progress through each decomposition stage and change. It is recognised that qualitative methods for estimating the PMI can be problematic due to the subjective nature of the descriptions of the decomposition stages, differences in training and experience between anthropologists and most importantly the highly variable process which is decomposition can affect the use of such descriptive PMI methods [1, 9, 11, 95-97].

A large number of researchers have examined the decay process in a variety of regions worldwide in an attempt to outline the decomposition process as it occurs in specific geographic regions, with the intention of using these stages to develop quantitative methods for PMI estimations, as the stages alone are not time regulated [1, 3, 18, 44, 49]. As of yet, no single standard protocol that can be applied universally has been created that exclusively estimates the PMI based on the observed decomposition stages. While many attempts have been made, limited

accuracy has been achieved specifically when addressing the extended PMI (once remains have dried or skeletonised) which is the purview of forensic anthropologists.

Landmark studies by Reed [21], Rodriguez and Bass [12] and Galloway et al. [16] (described above in Section 1.2) each investigated the decomposition process and created what is now referred to as the 'decomposition stages'. These studies approximated the time it took for a body to reach each stage of decomposition and the changes generally observed in each stage. This was to map the decay process of a body to assist in estimating the time since death in future death investigations when a body is discovered. Furthering the studies mentioned above, Rhine and Dawson [18] conducted a retrospective investigation into the decomposition process of remains in a region of New Mexico. They used the stages of decomposition outlined by Galloway et al. [16] and applied a point value ranging from one to fifteen based on the changes observed in an attempt to improve on the qualitative descriptions of decay by quantifying the decomposition process. Like Reed [21], Rodriguez and Bass [12], and Galloway et al. [16], Rhine and Dawson concluded that the environmental variables within the New Mexico region contributed to the varying decomposition sequences observed in their study [18]. They recommended that each geographic region worldwide needs to conduct their own decomposition studies to determine how a body decomposes within individual environments. Investigators must only then apply the decomposition data they receive for each region to estimate the PMI when a body is discovered.

In Spain in 2004, Prieto et al. [49] retrospectively analysed the relationship between observed decomposition changes and the PMI from photographs of human remains. The researchers categorised each body into one of six phases (putrefaction, initial skeletonisation, advanced skeletonisation, complete skeletonisation, mummification and saponification) based on the decomposition changes observed in the photographs. The extrinsic environmental features of the post-mortem scene were documented to identify the impact these variables and features may have on decay rates and, subsequently, PMI estimates. Time frames were outlined for each of the abovementioned phases based on how long it takes for a body to reach that phase and how long it remains within the phase. Prieto et al. [49] acknowledged that while certain factors such as temperature, insect activity and humidity were found to be the driving forces behind the decomposition process, as is comparable to many studies

worldwide, it is the immediate microclimate in which remains are discovered that is the major contributor to the condition and changes of remains.

Like Rhine and Dawson [18], Megyesi et al. [1] attempted to quantify the decomposition stages to estimate the PMI. They proposed a scoring protocol referred to as the 'Total Body Score' (TBS) which is based on the decomposition stages outlined by Galloway et al. [16]. A body is divided into three regions: (i) head and neck, (ii) trunk and (iii) limbs, and the changes observed on each body is assigned a point value according to the scoring protocol. The three values are summated to produce the final TBS. Megyesi et al. [1] used the degree of decay in conjunction with temperature, in the form of ADD, and created a regression equation with which the TBS is entered into, as a means to take a more empirical approach to estimating the PMI from the body itself. They found that 80% of the variation in decomposition was attributable to temperature (ADD) alone and suggest that by incorporating the state of decomposition of the body and ADD, the method has the potential to provide accurate PMI estimates. Megyesi et al. [1] also recommend the scoring protocol and equation is tested by researchers but advise that new equations may need to be developed which reflect local environments and climates more accurately.

Many researchers have attempted to validate the Megyesi et al. [1] formula in a variety of regions worldwide, all with varying results. However, each of these researchers concludes that decomposition is an environmentally specific process and methods need to be created for individual regions if an accurate determination of PMI is to be made.

In an attempt to improve PMI estimates for submerged human remains in the United Kingdom, Heaton et al. [44] retrospectively analysed photographs of decomposed human remains recovered from waterways. Heaton et al. [44] created a new decomposition scoring protocol which requires the investigator to assign a score to the body, based on how it 'best fits' into one of the outlined categories. The decomposition score is then entered into the given regression formula to estimate the ADD. To calculate the PMI, the average daily temperatures are summated until the predicted ADD is reached, thus, indicating an approximate time as to the length of time remains have been in the water. When Heaton et al.'s [44] method was validated by De Donno et al. [98] in Italy, they found a low correlation between the

scoring protocol and ADD and suggest that ADD alone cannot estimate the PMI and alternate variables must also be considered.

Also taking a statistical approach to time since death estimates, Vass [3] relied on twenty years of collected data and observations to create what he has coined a 'universal PMI formula'. Vass [3] identified four key variables found to greatly influence the decomposition process of remains found in Tennessee. They being, moisture, temperature, humidity and the ADD at which volatile fatty acids cease to be liberated from soft tissues. To quantify decomposition, Vass [3] asks the investigator to make a visual assessment of the observed state of decay and make an estimate of the percentage of soft tissue decomposition that the body has undergone. Vass [3] notes the method has successfully been applied in areas of Mid to Eastern United States, however, few studies have validated and reported on the accuracy and replicability of this method [99]. The studies which have applied the Vass formula suggest that the empirical values for ADD and moisture, which are provided by Vass in the equation, may be impacting on its accuracy when it is applied in different geographic regions [50].

Few decomposition studies within Australia have approached PMI estimates from an anthropological and taphonomic perspective. While many other decomposition studies have been undertaken, few have examined the decomposition process of a body and used it to estimate time since death in this unique climatic and environmental region.

Fitzgerald and Oxenham [20] examined the decomposition process in the Australian Capital Territory (ACT), in an attempt to quantify decomposition and create a method for estimating time since death in an Australian climate. They created a scoring protocol which requires the investigator to assign a point value from one to five based on the decomposition stage the body is observed to be in; where '1' is assigned for the fresh stage and '5' is assigned for the extreme decomposition stage. Fitzgerald and Oxenham [20] developed a regression equation to accompany the method to estimate the time since death based on the degree of decay observed and score assigned. This study acknowledged this method was created based on location specific temperature and humidity data but further studies should be undertaken to determine the effect of a temperate Australian climate on remains. Interestingly, they discovered a difference between the temperatures recorded by the data loggers at

their research site and the temperatures recorded by the local Bureau of Meteorology (BoM) weather station.

Expanding on Fitzgerald and Oxenham's [20] study, Marhoff et al. [2] examined the decomposition process in the Hawkesbury region, NSW, Australia and evaluated the replicability of Megyesi et al. [1] and Fitzgerald and Oxenham's [20] methods for PMI estimates to determine if they could accurately be applied. It was found that neither method could accurately determine the PMI, likely due to the change in environment and the small sample size used. However, the Megyesi et al. [1] method did show some potential to be used in PMI estimates of remains found in the Hawkesbury region. To better use the tested methods, Marhoff et al. [2] created alternate regression formulas for these methods from the data they collected, while still keeping the original protocols. It is unknown, however, if these alternate equations better estimate the PMI, as they have not yet been validated.

To date a standard method for PMI determination does not yet exist. As can be shown from the studies mentioned above, the field of forensic anthropology and taphonomy has highlighted the importance and need for the development of methods to accurately estimate the PMI of decomposed remains. Each of these studies concluded that the decomposition process is a geographically specific process requiring the development of regionally specific methods for PMI determinations. There is also no consensus, and perhaps some ambiguity, in the scoring or quantification of decomposition.

Currently, there is little in the literature addressing the decomposition process of remains in temperate Australia, specifically the Western Sydney region [2, 19, 20, 100, 101]. Fewer studies have attempted to create novel anthropological methods utilising the decomposition changes and taphonomic variables found in this unique environmental region or attempted to address the subjectiveness of current scoring protocols.

The present study will investigate the use of three currently published methods in the literature for PMI determinations to determine if they can be accurately applied in the Greater Western Sydney region. If the tested methods fail to accurately determine the PMI in this region, we aim to create a new anthropological method using the morphological changes occurring to remains in decomposition in conjunction with

various environmental variables in the hope of narrowing PMI estimates in the Western Sydney region and make quantifying decomposition more user-friendly.

1.7 Significance, aims and hypotheses of study

To date, no standard, universal formula exists to estimate the PMI based on the morphological changes a body undergoes during decomposition. Some anthropological methods have been proposed but have not yet been adequately investigated for their use as PMI methods in Greater Western Sydney.

The present study aims to examine and document the decomposition process of pig carcasses, as analogues for human remains, and provide information on how the summer and winter Australian climates, specifically of the Western Sydney region impact decay rates. It is unknown how this region, and specifically the winter and summer seasons, which experience extreme low and high temperatures during these seasons, respectively, affect decomposition. This study will, therefore, bridge this gap in the literature and provide forensic anthropologists and investigators with information that may be used in future death investigations.

In addition to documenting the decomposition process of remains within this region, the current study aims to evaluate the accuracy and replicability of three currently published methods in the literature that have been proposed to accurately determine the PMI of remains. They being, the protocol developed by Megyesi et al. [1] because it is commonly cited in the literature, the regression formula developed by Marhoff et al. [2] because it has not yet been validated and the PMI formula created by Vass [3] because of its 'universal' claim.

Finally, should the three evaluated methods fail to produce accurate estimations of PMI; we aim to develop a new quantitative method to narrow the estimations of PMI of decomposed remains, specific to the Western Sydney area. The objective is to narrow PMI estimations in the region from weeks and months to days.

We hypothesise:

❖ The warm weather associated with the summer months would accelerate the rate of soft tissue decomposition, resulting in a much shorter PMI when skeletonisation is complete than those findings found in the winter trials and reported in research performed in colder climates and seasons.

- ❖ Due to the climate specific nature of the decomposition process, the currently published methods for PMI estimates would incorrectly estimate the PMI or ADD of remains in the current study, suggesting these methods should not be applied in future estimations of PMI for remains found located in the Western Sydney region of eastern Australia.
- ❖ The newly created Marhoff-Beard method would produce more accurate predictions than those methods that have previously been developed internationally. Thus, this would provide a more appropriate model to use in a forensic setting to narrow down the time since death of remains found in the Greater Western Sydney area, with the potential to use this model across all temperate Australian climates.

The development of a method that can accurately estimate the PMI of decomposed remains within an Australian context will strengthen the integrity of this as evidence in court. The NSW Evidence Act 1995 Section 79 Subsection 1 requires expert witness testimony to be based on objective scientific standards whereby results can be reproduced. Validation of the reliability of anthropological methods could strengthen the justification for their use in PMI determinations, with the potential to use them as standard forensic protocols accepted by the Australian legal system.

Chapter Two

2   Materials and Methods

2.1   Fieldwork location

The field study was undertaken at Western Sydney University's Hawkesbury campus in Richmond, NSW (GPS coordinates: 33.61º S, 150.75º E). The location of the research site on Hawkesbury campus is an area comprised of dense bushland with an abundance of trees and grasses providing shade (Figure 2-1), and open grassy paddocks that receive direct sunlight during the day, as seen in Figure 2-2. The research site is isolated from the general public but livestock such as cattle were free to roam the area initially for Trial 1 until a fence was erected around the site. Periodically, cattle and sheep were given access to the field site for grazing. Wildlife such as kangaroos and foxes were also observed at the site.

Figure 2-1 Bushland at the Western Sydney University Hawkesbury campus research site depicting a shaded location.

The region commonly experiences hot summers and cool winters. The warmest month is January, with extreme heat waves that reach in excess of 40°C. Winter is

cool, with temperatures frequently dropping below 5°C. The coldest month is July. Despite being at the foot of the Blue Mountains the region does not experience snowfall like some of its neighbouring mountainous towns, however, it is subject to other extreme conditions such as heat waves, bushfires, severe wind and hail storms and flooding of low lying areas [102].

Figure 2-2 Grasslands at the Western Sydney University Hawkesbury campus research site depicting an open location.

2.2   Biological Materials

Eight domestic pig carcasses (*sus scrofa domesticus*) weighing approximately 60kg each were obtained post-mortem from the Hawkesbury Valley Meat Processors, Wilberforce, NSW for each of the four trials (thirty two pig carcasses in total). Carcasses were requested to be approximately 60kg as this is representative of an adult human and by obtaining similar size carcasses we were able to exclude body mass as a variable in the current study. All of the thirty-two carcasses obtained from the abattoir were slaughtered by a captive bolt to the head piercing the brain. This method is standard practice for the slaughter of animals in abattoirs.

Pig remains are commonly used as an analogue for human cadavers in studies of decomposition as they share many important biological similarities, namely the

thickness of the skin, organ and tissue structure, degree of body hair and the type and presence of gut microbiota [67, 103-109]. The similarity of these particular characteristics makes pig carcasses ideal analogues for decomposition research.

Upon delivery at the research site, each set of remains was placed on their side on the soil surface in their designated location (e.g. sun or shade). Remains were enclosed within a metal cage to prevent vertebrate scavenging, while still being exposed to insect activity and the elements, as seen in Figure 2-3.

Figure 2-3 An example of the metal cages protecting the pig carcasses from vertebrate scavenging.

At the beginning of each new trial, the cages were thoroughly washed to remove any remaining decomposition by-products and bacteria from prior studies and then placed over the new carcasses. The previous remains were then covered in heavy duty chicken wire so that the decomposition process could continue to be observed without large vertebrates disturbing the carcasses.

2.3   Climatic Data

Two Tiny Tag Plus 2 data loggers (Figure 2-4) recorded temperature data hourly for the length of the study (three months per trial). The two data loggers were attached to the side of two different cages using cable ties. One data logger was always attached

to a cage that was situated in direct sunlight during the day and the other data logger was attached to a cage that was positioned under the shaded canopy of trees. Situating the data loggers in two separate locations of the research site allowed for the study of carcasses located in the sun and the study of carcasses located in a shaded environment, to examine if there was a difference in the decomposition process between microclimates. It also allowed for any change in the microclimates to be accounted for.

Figure 2-4 Tiny Tag Plus 2 data logger attached to one of the cages to measure temperature data at the research site.

Previous studies have suggested that temperatures at freezing or below retard the biological processes triggering decomposition and arthropod activity diminishes [1]. As such, temperature dependant methods, such as Megyesi et al. [1] and Marhoff et al. [2], will not use temperatures below 0°C in their PMI calculations, with any temperatures below this temperature recorded simply as zero. This method was adopted for the present study also, to remain comparable to Megyesi et al. [1].

Temperature was also obtained from the local weather station for the Bureau of Meteorology (BoM) at the Royal Australian Air Force (RAAF) base in Richmond, NSW, approximately 4.7km from the research site. This was to determine if there was a significant difference between the microclimate of the field site and the temperatures reported by the BoM for the overall Western Sydney region. Weather

data such as rainfall levels, average daily humidity levels and wind speed was also obtained from the BoM for analysis.

2.4 Experimental Design

Over an 18 month period from June 2014 to March 2016, four experimental trials were undertaken with each trial running for a period of 90 days. On the 10 June 2014 (Trial 1), 09 December 2014 (Trial 2), 10 June 2015 (Trial 3) and 08 December 2015 (Trial 4), at approximately 2pm on each of the above dates, eight adult pig carcasses were slaughtered and collected from Hawkesbury Valley Meat Packing, Wilberforce, NSW. For each of the abovementioned trials, the carcasses were transported to the research site on Western Sydney University's Hawkesbury campus via a trailer and were covered by a standard, blue tarp for the 20 minute trip (approximately 15.5km) to the research site. Upon arrival at the site, carcasses were placed in their designated places, as seen in Figure 2-5, and caged (Figure 2-3). The date the remains were placed at the site (see above) was coded as 'Day 0' for the purpose of PMI calculations.

Each set of remains for each of the four trials were placed approximately one metre apart from one another, in a low lying grassed area to naturally decompose on an environmental surface common throughout the Western Sydney region. For each trial, four of the eight pig carcasses were placed in an area under the canopy of trees (Figure 2-1) and received shade for the majority of the day. The other four pigs were placed in areas where they received exposure to direct sunlight throughout most of the day (Figure 2-2). Overall, this resulted in sixteen shaded carcasses and sixteen sun exposed carcasses in total during this study. Photographs were taken once the remains had been deposited to begin documenting the decomposition process. The average daily temperatures for the 10 June 2014 and 09 December 2014 were 15°C and 25°C respectively, with light showers throughout the day, as such; the ground at the research site was damp where the carcasses were placed. For 10 June 2015 and 08 December 2015, the conditions were dry and temperatures were similar to the above trials, namely 15°C for the June placement day and 25°C for the December carcass placement day.
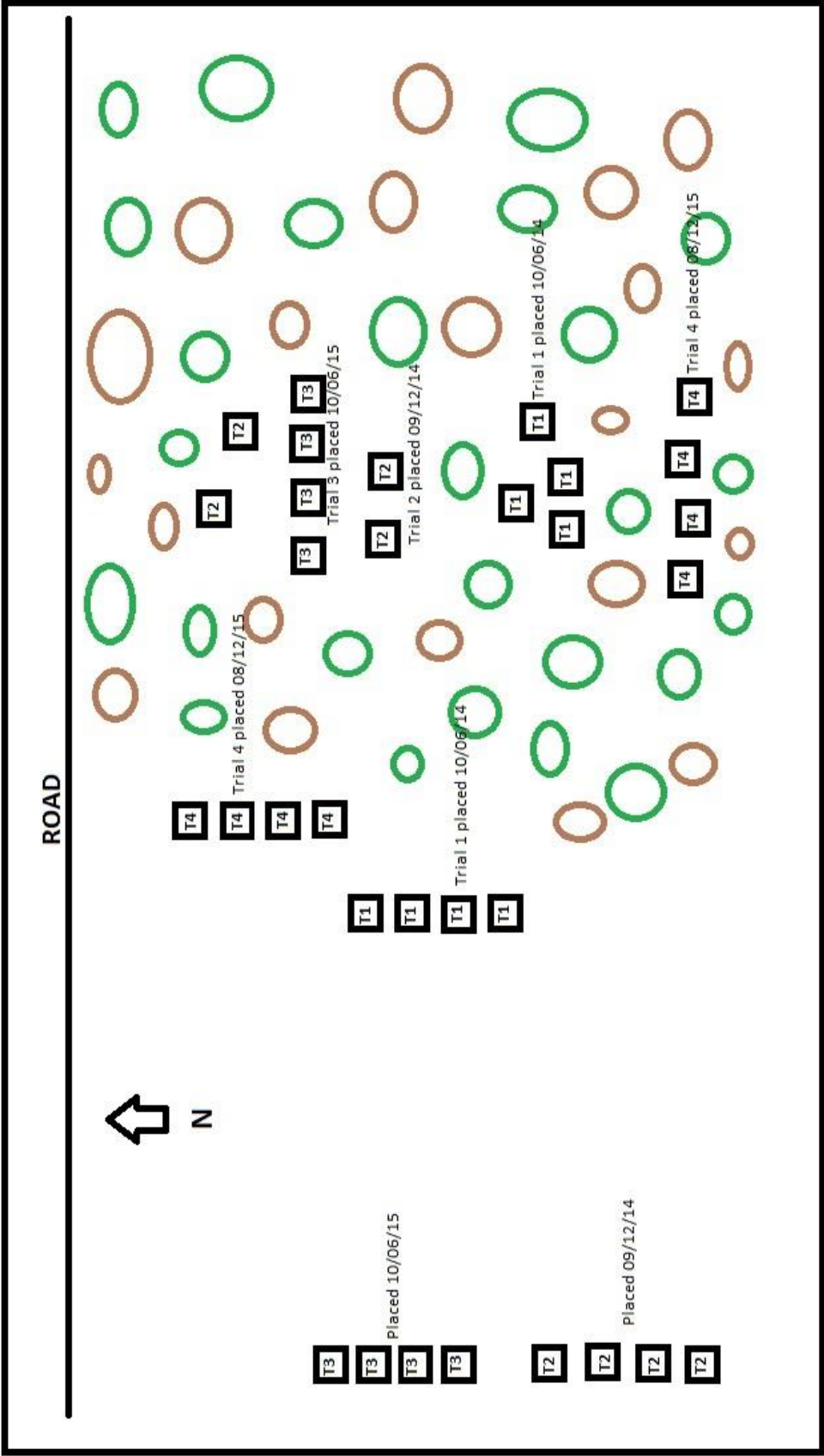
Figure 2-5 Schematic map of the decomposition field site at Western Sydney University's Hawkesbury campus. T1, T2, T3 and T4 represent the four trials and the location of the carcasses for Trial 1 through Trial 4. The green and brown circles indicate trees and shrubs. Map is not to scale

Data collection was undertaken twice a week, every Monday and Thursday at approximately 9am, for carcasses in all four of the trials, except for the first three weeks of both summer trials, when data was collected daily due to the rapid progression of decomposition. Visual observations of the remains and photographs were taken at the time of data collection for record keeping purposes. Images taken on each sampling day were photographed using a Samsung NX2000 digital SLR camera with a 20-50mm lens to document the decomposition changes.

Initial observations such as the description of decay and general weather conditions, such as, temperature, rainfall, humidity and wind speed were documented upon arrival to the site on each data collection day. While the various species of insects were not of interest to this project, the general presence of insect activity and their abundance or lack thereof was noted as it is recognised as a taphonomic agent. The degree of decomposition for each of the carcasses was then scored for the TBS using the protocol published by Megyesi et al [1] and the percentage of decomposition was recorded following the guidelines outlined by Vass [3].

As this study was an observational study only, no biological samples were collected for examination. Each trial ran for a period of 90 days, with the final day for data collection of each trial being 10 September 2014 (Trial 1), 09 March 2015 (Trial 2), 10 September 2015 (Trial 3) and 08 March 2016 for Trial 4.

To calculate the PMI of the carcasses used throughout the four trials, the protocol by Megyesi et al. [1] using ADD, the regression equation by Marhoff et al. [2] which also makes use of the temperature variable in the form of ADD and the Universal PMI formula by Vass [3] utilising a variety of variables such as weather data and the percentage of soft tissue decay, were employed in this study. These three methods are explained in detail below.

2.5 The Megyesi et al. method

The observed state of decomposition each carcass demonstrated was first scored using the scoring protocol by Megyesi et al. [1]. The carcass was divided into three anatomical regions (head and neck, trunk, and limbs) as shown in Figure 2-6 and a score was assigned based on the most advanced stage of decay observed in each of the three anatomical regions according to the scoring protocol (Appendix A) [1]. Due to the lack of uniformity in the decomposition process throughout a body [1,

20], these three areas are scored independently from one another and those scores are then combined to give a Total Body Score (TBS). The TBS represents the overall decomposition stage presented by a set of remains.
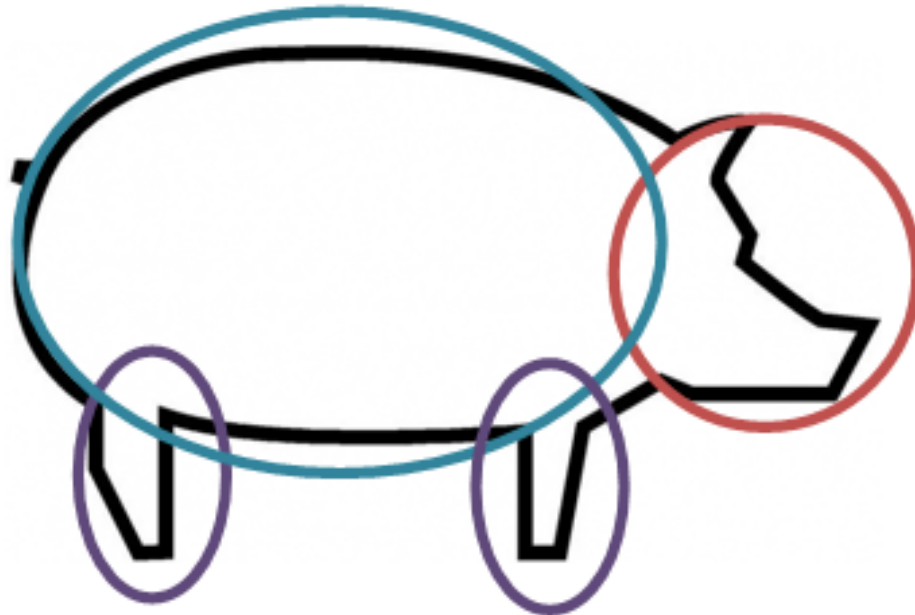
Figure 2-6 Division of the pig carcass for scoring the TBS, where blue is the trunk, red is the head and purple is the limbs. Adapted from Megyesi et al. (2005).

For example, if the head and neck of the remains showed mummification with bone exposure on less than one half of the area being scored, the trunk also exhibited mummification with bone exposure on less than one half of the area being scored, and the limbs showed mummification with bone exposure on less than one half of the area being scored, then the following scores would be allocated: head and neck- 9, trunk- 8 and limbs- 7. This would result in a TBS value of 24 out of a possible 35. These individual scores suggest that overall this particular set of remains had mummified. Whilst each of the individual regions received the same description of decomposition, each of the TBS values was different. This is due to the difference in decomposition changes which occur to the various regions of the body [1].

At the completion of each trial and subsequent data collection, the final TBS value was placed into Equation 1 (below) to determine the estimated ADD it would take to reach this TBS score or stage of decomposition. This was repeated for all eight carcasses in each of the four trials.

Equation 1: ADD= Log 10 (0.002*TBS*TBS+1.81) ±388.16

where 388.16 is the standard error for regression found by Megyesi and colleagues in the original study [1]. The value generated by this equation is the estimated or predicted number of ADD's that is required for the remains to reach the stage of decomposition as described by the allotted TBS when using the scoring protocol by Megyesi et al. [1]. Working backward from the date of discovery of remains, or in the case of this project, the completion of each trial, the average daily temperatures are summated until the ADD value generated by Equation 1 is reached. The PMI is the day the sum of Equation 1 is reached. For example, using the TBS example value above (TBS=24) and the date of discovery as the 8th September, estimating ADD and the time since death occurs as follows:

ADD=Log 10 (0.002*24^2+1.81) ±388.16

ADD= Log 10 (2.69) ±388.16

ADD= 912.01±388.16

Estimated ADD= 523.85 days °C to 1300.17 days °C

Using the average daily temperatures recorded by the onsite Tiny Tag Plus data loggers and counting backward from the 8th of September 2014 (this was the conclusion of Trial 1 Winter), it would take approximately 54 days for a value of 912.01 days °C to be reached, and when including the error range above, a PMI range between 30 to 81 days. Therefore, this method is estimating the date of death to be the 16th July 2014, or when expressed as a range the date of death is between the 20 June 2014 to 10 August 2014.

The actual or true ADD in the current study is calculated by retrospectively summating the average daily temperatures from the date in which the various trials concluded.

2.6 The Marhoff et al. Formula

The Marhoff et al. [2] equation for estimating time since death was developed from a preliminary study conducted in the Hawkesbury region in 2013. This regression equation was developed from the results obtained in the 2013 study as an alternative method for determining PMI in an Australian temperate environment based on the Megyesi et al. model [1]. Because the Megyesi et al. [1] ADD method did not

perform accurately when estimating PMI in the Marhoff et al. [2] study a new equation was developed to be more accurate and specific for the region [2]. This method follows the same protocol as the original Megyesi et al. [1] method where remains are divided into the three body regions seen in Figure 2-6 and each region given a TBS based on the scoring protocol outlined by Megyesi et al. (Appendix A) [1]. The three TBS values for the different regions are summated to produce the final TBS to be included in the equation. This equation is as follows:

Equation 2 Marhoff et al. ADD= -85.8+39.7(TBS)

The value produced by the above equation is the estimated ADD that is required for the remains to reach the observed stage of decomposition, as described in the scoring protocol and assigned TBS created by Megyesi et al.[1]

As with the Megyesi et al. [1] method, using the ADD value generated by Equation 2, the researcher works backward from the date of discovery of the remains summating the average daily temperatures recorded for the region until the value generated by the equation above is reached. The day the value produced by Equation 2 is reached, is the PMI or date of death of the remains.

2.7   The Vass Universal Post-Mortem Interval Formula

The observer first takes into account the entire body and makes a visual assessment of how much of the body has undergone decomposition. The percentage of soft tissue decomposition was first determined based on the researchers' observations of the carcasses and was expressed as a decimal (e.g. 30%= 0.3). To better determine the percentage of decay, the table accompanying this method which correlates the decomposition stages (i.e. fresh, bloat) with a decomposition percentage range, was used. The correlation also depends on whether the average daily temperature for the region was less than or greater than 12°C. The <12°C ranges were used during the winter trials as the average daily temperatures fell below this value and during the summer trial the >12°C was used as temperatures always exceeded this value. Temperature is expressed as degrees Celsius.

Throughout each of the trials the average daily temperatures and average humidity levels were recorded. The average daily temperatures (expressed as follows e.g. 20°C=20), the average humidity levels (expressed as follows e.g. 50%=50) and the percentage of soft tissue decomposition were placed into Equation 3 (below) upon

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the conclusion of the trial periods to estimate the PMI in days. All other values in the equation are constants that have been empirically derived from decades of research [3].

Equation 3 PMI $_{aerobic}$=1285*(decomposition/100)/0.0103*temperature*humidity

For example, using the percentage of decomposition example as 55% with average daily temperatures of 11.69°C and average humidity levels of 63.33% the following PMI of a set of remains as follows:

$PMI_{aerobic}$= 1285*(decomposition/100)/0.0103*temperature*humidity

$PMI_{aerobic}$=1285*(55/100)/0.0103*11.69*63.33

$PMI_{aerobic}$=706.75/7.62

$PMI_{aerobic}$= 92.74 days

Once calculated, subtract 92.74 days from the date the remains were discovered to determine the date of death.

2.8 Statistical Analysis

All analyses were performed using the software Statistical Package for Social Sciences (SPSS version 23.0). To produce the most accurate results, it first needed to be determined which set of temperature data (i.e. onsite data loggers versus the BoM weather station) should be used to perform analyses that included temperature data. To determine if a statistical difference existed between the temperature data recorded by the onsite date loggers at the research site and the temperature data recorded by the BoM, a paired *t*-test was performed. If a *p*-value greater than 0.05 was found, then there was no significant difference between the temperatures recorded by the two mediums. If a *p*-value less than 0.05 was achieved, then there was a statistically significant difference between the temperatures reported by the BoM and the Tiny Tag data loggers at the field site. If a significant difference was found, then the temperatures recorded by the data loggers at the research site would be used for all analyses involving the temperature variable as it more accurately depicted the climatic condition the remains were exposed to.

As a means to assess the patterns of decomposition for each carcass, scatter plots were created to demonstrate the relationship between (i) TBS and the actual PMI, (ii)

the percentage of soft tissue decomposition and the actual PMI. Using Equation 1 [1], Equation 2 [2], and Equation 3 [3] described above, the values predicted when applying these equations were plotted against the known ADD and PMI recorded throughout the four trials. If the protocols applied were correctly estimating the ADD and PMI of the remains in this study, then all values should lie along the x=y line, suggesting a linear and equal relationship. This was confirmed with an r^2 value.

Should the currently published methods be unable to accurately estimate the ADD or PMI under the current experimental conditions, a new method for PMI calculations will be developed using regression analysis and linear mixed modelling. The new method will then be validated retrospectively on pig models and a human study.

Chapter Three

3 Results- Morphological Changes of the Winter Trials

3.1 Introduction

This chapter explores the gross morphological changes observed on decomposing pig remains over two winter periods: Trial 1 (2014) and Trial 3 (2015). Each trial was conducted over a 12-week period, with both trials running June 10th to September 10th (2014 and 2015). Each trial observed decomposition processes on eight carcasses, with a total of sixteen carcasses across the two trials. During the trials, four carcasses were placed in a shaded environment and four carcasses were placed in a full sun exposed environment to assess the effect of different microclimates on the decomposition process. Descriptions of the morphological changes commonly observed over the 12-week winter trials were recorded according to standard decomposition stages as published by Megyesi et al. [1] and Galloway et al. [16], and are described in Chapter 1. Table 3-1 is a comparison of the average time taken for remains from both winter trials to commence each stage of decomposition as described by Galloway et al. [16] and Megyesi et al. [1]. Time taken is measured in days since known date of death (Day 0).

Table 3-1 Comparison of the winter trials when remains entered the various decomposition stages, as described by Megyesi et al. [1] and Galloway et al. [13].

Stage	Trial 1 2014 (winter)	Trial 3 2015 (winter)
Fresh	Day 0	Day 0
Early decomposition	Day 6	Day 7
Advanced decomposition	Day 21	Day 49
Skeletonisation	Not reached	Not reached
End of trial	Day 90	Day 90

3.2 Trial 1 (winter, 2014)

Figure 3-1 summarises the major soft tissue changes observed during the winter trial of 2014. All carcasses progressed through fresh (Figure 3-1a), early (Figure 3-1b-c) and advanced (Figure 3-1d-e) decomposition stages. The final stage, skeletonisation,

was not reached, with carcasses instead becoming mummified (see Chapter 11 Glossary) during the advanced decomposition stage (Figure 3-1f). Details of specific observable changes within each decomposition stage are presented below.

Figure 3-1 Demonstrative photographs of a carcass in the shaded microclimate taken during Trial 1 (winter) 2014 a) fresh, no visible changes b) first colour change, green discolouration at lower abdomen c) bloat with colour change moving from green to dark green/black d) formation of maggot masses and cadaveric island, skin slip first observed e) carcasses beginning to dry f) mummified.

3.2.1 The Fresh Stage

From the time the pigs were killed on June 10th 2014 (Day 0; Figure 3-1a), the fresh stage (refer to Chapter 1 Section 1.2) commenced and continued until approximately Day 6. Minimal invertebrate activity was observed initially with some spiders, ants and flies present and this was consistent for both winter trials. The species of the

insects and arachnids are unknown, as this was outside the scope of the present study, which focused on only visual documentation of soft tissue changes. During this stage lividity and marbling became apparent on the legs and abdomen of all eight carcasses on Day 2 (Figure 3-2). A green discolouration (Figure 3-1b) and bloating of the carcasses on Day 6 (Figure 3-1c) signalled the end of the fresh stage and the commencement of the next stage, known as 'Early Decomposition'.
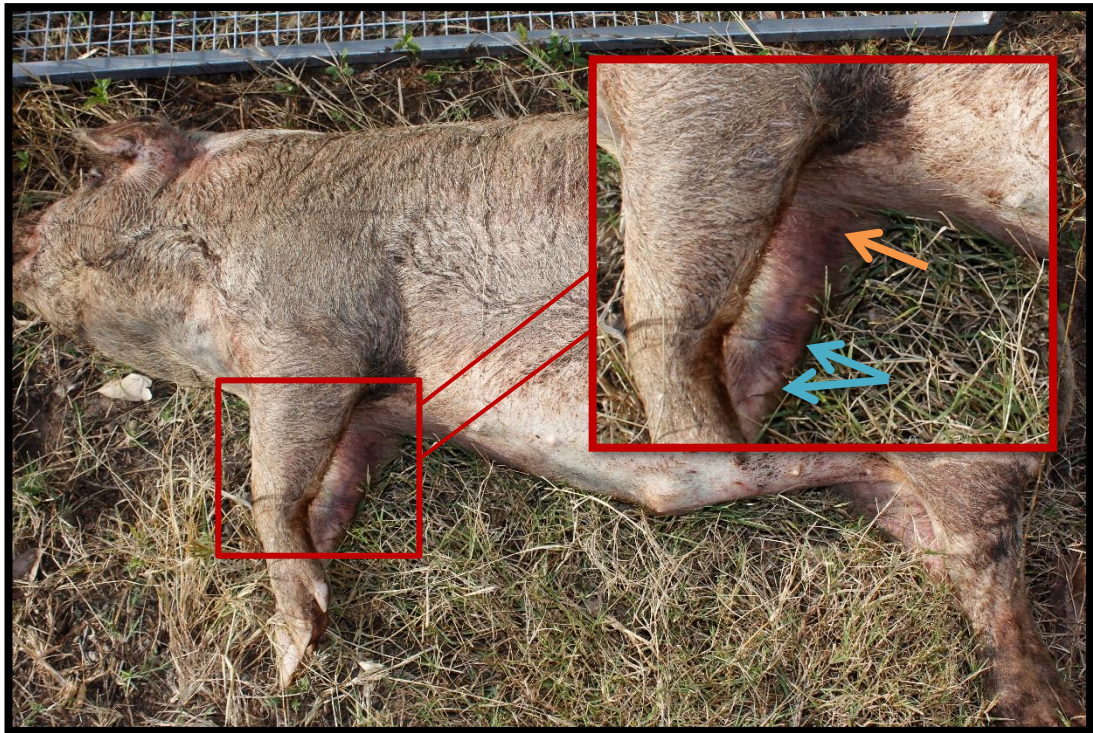
Figure 3-2 Representative carcass demonstrating lividity (inset, orange arrow) and marbling (inset, blue arrows) observed on the fore limbs during the fresh stage of decomposition

3.2.2 Early Decomposition Stage

The 'early decomposition' stage for this trial commenced on Day 6, and lasted for approximately 10 days until Day 16. During this period, it was observed that all eight carcasses demonstrated a green discolouration beginning in the lower right abdomen (right iliac fossa) above the caecum where bacteria is most concentrated in this area of the abdomen (Figure 3-1c) [58, 87], before spreading cranially along the length of the trunk towards the neck and head and finally, the limbs.

All carcasses were observed to have reached a bloated state by Day 9 of the trial, with bloating occurring to all body regions (head, trunk, limbs) and all demonstrated a green-black discolouration simultaneously to bloating (Figure 3-1c and Figure 3-3).

Maggot activity was present in all carcasses on Day 13, first beginning in the mouth and head before progressing to the legs, neck, rear and abdomen (Figure 3-4). Carcasses located in the shade demonstrated a greater amount of maggot activity than carcasses located in the sun but carcasses in the sun had beetle activity which was not observed on remains in the shade.

Figure 3-3 Representative carcass (sun) demonstrating a green-black discolouration that appeared on all carcasses simultaneous to bloating. This discolouration extended from the abdomen, along the trunk and finally to the head and neck region.

Figure 3-4 The presence of maggot activity as observed on Day 13. Figure 3-4a demonstrates the level of maggot activity available in the mouth of shaded carcasses. Figure 3-4b demonstrates the maggot masses present in the mouth of the sun exposed carcasses.

One sun exposed carcass was observed to be decomposing at an accelerated rate compared to the other carcasses by Day 13. On this day, these remains demonstrated a blackened abdomen and the first signs of adipocere near the neck region (Figure 3-5). Three other sets of remains were also observed to develop adipocere during this trial, but this was not observed until Day 21 for one sun exposed carcass and Day 68 for the other two remains (one sun exposed and one shaded).
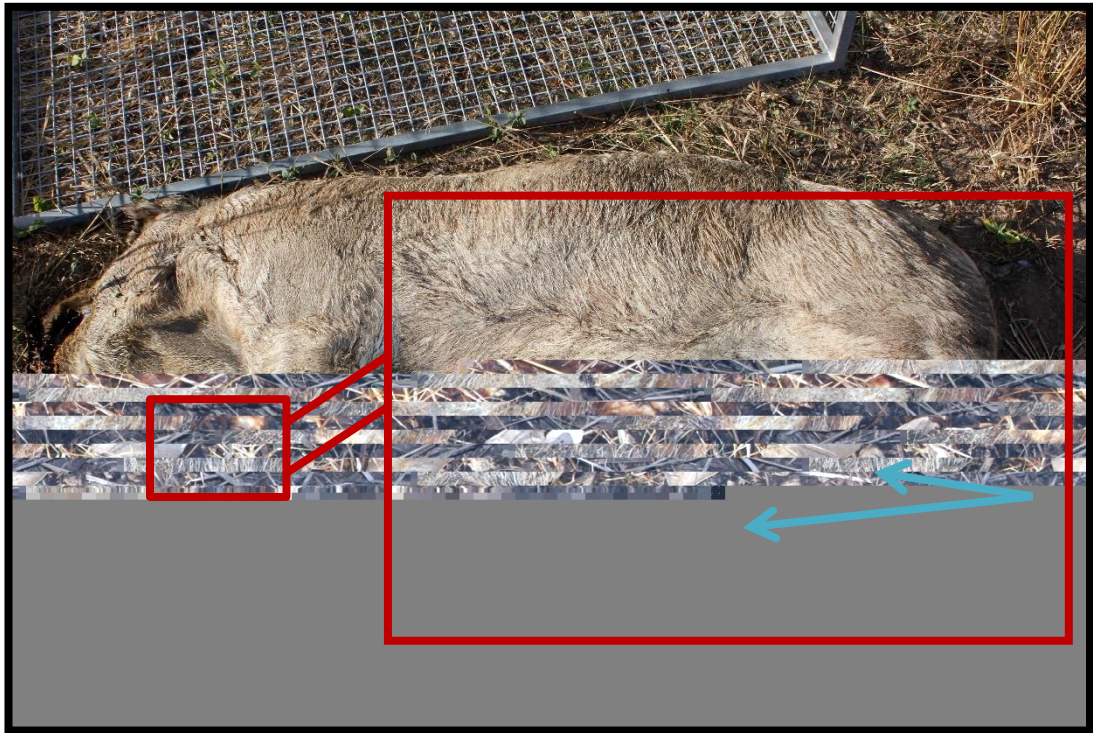
Figure 3-5 The first sign of adipocere formation (inset, blue arrows) near the neck of a sun exposed carcass which was observed to be decomposing at an accelerated rate to all other remains in Trial 1.

Between Days 16-21, three out of eight carcasses demonstrated transitional features of both the early and advanced decomposition stages, with minimal bone exposure of the face and skull observed. This feature is typically classified as being a feature of the advanced decay stage [16].

On Day 16, the first sign of bone exposure on the superior surface of the skull was observed on one of the eight carcasses. At this time point, it was observed that this shaded carcass had the most extensive maggot activity of all eight individuals. Bone exposure was not observed on any other individual until Day 21, when the bones of the hard palate and maxilla became visible on two additional sun exposed carcasses.

the remainder of the study (Figure 3-7). The skin of the legs and neck became black and papery by this time point for all other carcasses and the soft tissues of the remaining body regions began to blacken and dry.

Day 44 also saw no visible maggot activity on the external surface of any of the eight remains; however, beetles were more apparent at this time point, mostly on the sun exposed carcasses. The species of beetle was not identified as this was outside the scope of the current study, however, a previous study at this site in 2012 [93], listed beetles from the histerid, silphidae and trogidae family, as being present during this stage of decomposition.

Figure 3-7 Representative carcass demonstrating the lack of change for one carcass (shade) at Day 44 (Figure 3-7a), Day 61 (Figure 3-7b) and at the end of the trial at Day 90 (Figure 3-7c).

Decomposition slowed substantially for all carcasses from Day 41 through to Day 44 with no further changes observed until Day 68. After a period of heavy rain from Day 68-80, remains had paled in colour (**Error! Reference source not found.**). A dipocere continued to form on Pig 6 (sun) and was also visible on the sun exposed Pig 7 (hind legs) and the trunk of Pig 3 (shaded). During this period of heavy rain, carcasses displayed increased bone exposure, particularly of the hind leg bones.

By Day 79, the abdomen of the eight carcasses had lost all internal integrity collapsing completely. Bone exposure was observed at the shoulder region and the front legs of two of the carcasses. No further observable soft tissue changes were discerned between Day 79 until the conclusion of the trial on Day 90. At the conclusion of the trial, all carcasses had completely dried, demonstating the characteristic hardened exterior common to mummified remains (Figure 3-1f and Figure 3-9) [87].

Figure 3-8 a) Shaded carcass before the rainfall at Day 44 to show the natural colouring/pigmentation of a pig b) After a period of rainfall, remains paled in colour and lost biomass as can be seen on the shaded carcass (Trial 1: winter, 2014).

Figure 3-9 Representative image of the abdomen of a carcass in the full sun microclimate depicts the characteristic features of mummification (blue arrows). Remains did not decompose further once mummification occurred.

3.2.4   Trial 1 (winter) – Summary of the main findings

In Trial 1, all carcasses progressed through to the early decomposition stage by Day 21 and achieved mummification during the advanced decomposition stage by Day 60; however the rate that each individual carcass progressed through each stage and the soft tissue changes that were observed was highly variable.

Initially, the sun exposed carcasses, which were located in an area with direct sun exposure during the day, decomposed more rapidly. One sun exposed carcass decomposed the slowest of all sets of remains with few observable changes being noted except for bloating, sinking and drying of the remains. However, given that it was the smallest carcass, it lost biomass quickly unlike the other carcasses which maintained internal structural integrity for a longer period of time.

One sun exposed and one shaded carcass decomposed at an accelerated rate compared to the other sets of remains; however, they did not decompose in a similar fashion. One sun exposed carcass exhibited morphological changes early and rapidly in the trial and was the only carcass to exhibit substantial formation of adipocere. Initially, few changes were observed on a shaded carcass but when decomposition began, the outer soft tissues soon desiccated, internal integrity was lost and the

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remains underwent no further changes through to the completion of the trial at Day 90. While all other remains decomposed differently, the differences were not as dramatic as those changes reported for the above carcasses.

All carcasses reached a stage of preservation with little to no skeletonisation observed.

3.3 Trial 3 (winter, 2015)

Figure 3-10 summarises the major soft tissue changes observed during the winter trial of 2015. As observed in Trial 1, skeletonisation was not reached in any carcass in this trial, with remains desiccating instead (Figure 3-10f). Details of the soft tissue changes observed during the major stages of decomposition during this trial are discussed below.

Figure 3-10 Demonstrative photographs of pig carcasses taken during Trial 3 (winter) 2015 a) fresh, no visible changes b) early decomposition; first colour change, green discolouration at lower abdomen c) early decomposition; formation of maggot masses, cadaveric island, first bone exposure d) advanced decomposition; sinking of the abdomen due to loss of biomass and structural integrity e) advanced decomposition; drying of the carcasses.

3.3.1 The Fresh stage

The fresh stage began on the date of death and lasted approximately 7 days (Figure 3-10). As observed previously in Trial 1 (winter), minimal invertebrate activity was observed initially with some spider, ant and flies present. During this stage, autolysis commenced internally with the first visual signs of autolysis were observed as lividity and marbling on the thorax and fore limbs of all eight carcasses on Day 5 of Trial 3. A green discolouration and bloating of the carcasses on Day 7 of Trial 3

marked the end of the fresh stage, indicating the remains had progressed into the next decomposition stage: the early decomposition stage [16].

3.3.2 The Early Decomposition stage

Early decomposition commenced on Day 7 of the trial and did not conclude until Day 49. On Day 7, a green hue was observed around the face of each of the carcasses. Each of the four sun exposed carcasses showed very early signs of bloat at approximately Day 10. The carcasses in the shade began displaying signs of bloat approximately 10 days later.

All remains in Trial 3 (winter) demonstrated the most dramatic taphonomic changes by Day 20. The eight carcasses had a green discolouration on various body regions such as the lower abdomen, face and forelimbs, with decomposition fluid beginning to purge from the mouth of each carcass (Figure 3-10b). Full bloat of the entire body (head, trunk and limbs) and maggot masses of the mouth was observed in three carcasses (two in the sun, one in the shade). The remaining five carcasses only reached a partially bloated state and were beginning to display signs of sagging of the lower abdomen (Figure 3-11). The mouth, snout, ears and eyes had begun to blacken and dry out for all sets of remains.

Figure 3-11 Representative carcass (shade) demonstrating sagging of the abdomen (blue arrow), maggot masses at the mouth and purging of decomposition fluid from the mouth and snout (red arrow).

In comparison to the previous two trials of this study (Trial 1 (winter) discussed above, and Trial 2 (summer) to be discussed in Chapter 4), external soft tissue decomposition was very slow in the beginning of this trial, with visual signs of soft tissue decay not observable until Day 26.

The soft tissue of the lower abdominal region began to decompose first, followed by the head region in all carcasses by Day 30 (Figure 3-10c). By Day 34, active decomposition was observed in most carcasses, which resulted in the skin of the trunk closest to the ground splitting where maggots had consumed the underlying soft tissues. Two of the full sun exposed carcasses were darkening in colour at this time point to a dark brown/black discolouration but otherwise continued to decompose slightly slower than the other sets of remains.

Day 42 of this trial saw extensive maggot activity in each of the eight carcasses. All sets of remains had moist decomposition of the soft tissues of the mouth, snout, lower abdomen/hind leg junction. Due to the great number of maggots consuming the tissues of the carcasses, substantial amounts of liquefaction occurred from the various orifices (Figure 3-12). Two carcasses located in the sun and shade respectively, began to show small but initial signs of bone exposure in the fore limbs, suggesting an overlap of the early and advanced decomposition stages, with a shaded carcass appearing to be the most decomposed carcass of the eight carcasses (Figure 3-10d). Overall by Day 42, carcasses were decomposing at a very similar rate, a phenomenon not seen in the previous winter trial.
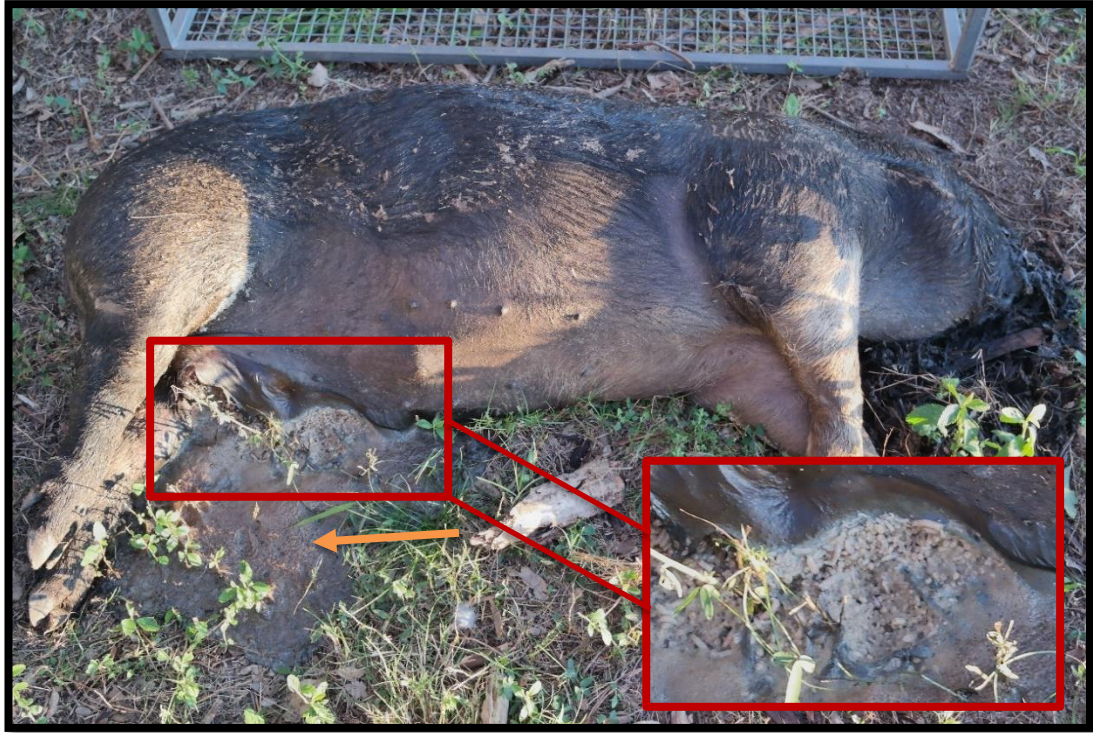
Figure 3-12 Representative carcass (shaded) in Trial 3 (winter), Day 42, demonstrating maggot masses at the lower abdomen and hind leg junction (inset), with large amounts of liquefaction purging from this region (as indicated by the orange arrow).

3.3.3 Advanced Decomposition stage

The Trial 3 (winter) 2015 remains entered the advanced decomposition stage at approximately Day 49. Carcasses were beginning to dry and developed a black discolouration overall. The large maggot masses previously observed had subsided but some maggots were still present, particularly in the abdominal region.

Due to skin slippage and soft tissue decomposition, the facial features of each of the carcasses were now almost unrecognisable. Each set of remains demonstrated bone exposure of the jaw bones (maxilla and mandible) and a shaded carcass was decomposing at an accelerated rate compared to the other seven remains (Figure 3-10e and Figure 3-13).

Figure 3-13 Comparison of two carcasses at Day 49. Figure 3-13a carcass (sun) has not yet dried, tissues are soft and moist, abdomen caved in. Figure 3-13b shows the loss of structural integrity and biomass of a shaded carcass. The remains have sunk, are beginning to dry and display some bone exposure of the fore limbs (as indicated by the red arrow).

On Day 54, bone exposure of the skull and legs had become more prominent on each of the carcasses, with the exposed bones of the legs demonstrating a dry, yellowed

exterior. Maggot masses were no longer observed at this point but may have been residing in the internal cavities of the carcasses for warmth.

Remains demonstrated fewer observable or significant taphonomic changes by Day 70. Three of the four carcasses in the shade had reached desiccation, while the majority of the full sun carcasses, retained a moist, flesh-like appearance and did not show any observable signs of drying particularly at the abdomen and head regions.

Day 76 marked the beginning of a two-week rainfall period. All carcasses rehydrated due to the rainfall, giving the carcasses a moist appearance (Figure 3-14), however, active decomposition did not resume and they quickly dried once the rain had ceased (Day 90) (Figure 3-10f and Figure 3-15). Carcasses displayed no further taphonomic changes after the trial finished.

Figure 3-14 a) an example of a sun exposed carcass prior to a period of rainfall, whereby this carcasses is in the process of drying b) remains have rehydrated and taken on a moist, fresh appearance after the rainfall.

Figure 3-15 Representative shaded carcass demonstrating the soft tissues drying out after a 14 day period of rainfall from Day 76 (Trial 3: winter, 2015).

3.3.4 Trial 3 (winter) - Summary of the main findings

All carcasses in Trial 3 (winter) decomposed at a similar rate to one another, despite the difference in microclimate between the carcasses. The decomposition rate of the remains through the early decomposition stage in Trial 3 was much slower than the previous trial (Trial 1), taking approximately 42 days (Table 3-1). The early decomposition stage also commenced later at Day 7 in Trial 3 (Day 6 in 2014) and the advanced decomposition stage commenced at Day 49 in 2015. All carcasses remained in these aforementioned stages for a longer period of time before progressing to the next decomposition stage.

With the rainfall levels experienced during this trial (Appendix D), remains appeared to rehydrate but did not continue to actively decompose. They dried quickly and then remained in this state until the conclusion of the trial.

Bone exposure was first observed at Day 49, when remains entered the advanced decomposition stage, with all eight carcasses displaying exposure of the jaw.

3.4   Discussion

3.4.1   Differences between the two winter trials

Maggot activity was first observed at Day 20 in Trial 1 but much earlier at Day 13 in Trial 3. The presence of adipocere was observed in Trial 1 in two different sets of remains, however, was not detected in Trial 3 despite similar conditions between the two trials during the early decomposition stage, when adipocere was first observed. Komar and Beattie [110] did not observe maggot masses on some of their medium and large sized pig carcasses (medium: 36-80kg; large: 156-162kg) until Day 15 of their trial and suggest the colonisation of insects to remains may be dependent on the size of the carcass, as smaller carcasses within their study (approximately 19-26kg) demonstrated maggot masses on Day 5. All pigs used in the present study were approximately 60kg (see Chapter 2).

The remains in Trial 3 (winter) were in the early decomposition stage longer than the remains in Trial 1 (winter). The remains in Trial 1 (winter) entered the advanced decomposition stage at Day 21, whereas the remains in Trial 3 (winter) did not progress to the advanced stage until approximately Day 49.

Day 44 marked the beginning of the desiccation process for all sets of remains in Trial 1, a finding comparable to Komar and Beattie's [110] study conducted in Edmonton, Canada. This varied greatly to Trial 3, where this process did not begin until Day 69 for all carcasses. At the conclusion of each trial at Day 90, all carcasses appeared as a hard, outer shell overlying the skeletal structure.

The presence of adipocere was also only observed in one of the winter trials undertaken; Trial 1. Adipocere was observed on three different sets of remains at varying times in this trial, all of which were located in the sun. It is not uncommon for adipocere to present at varying times and on only certain carcasses during a trial, as found by Wilson [104]. In the present study, adipocere was first observed at Day 13 and first appeared at the neck, followed by the lower abdomen and finally along the back of these carcasses, however, adipocere formation was not observed on any carcass in Trial 3.

3.4.2 Similarities between the two winter trials

3.4.2.1 The Fresh stage

All remains in both winter trials remained in the fresh stage for a similar timeframe, approximately 6-7 days. This stage saw the progression of some invertebrate activity from ants, spiders and flies, in order of progression, to the sixteen carcasses used in these two trials. While the presence of lividity and marbling occurred at different times across the trials, these early characteristic changes were first observed along the thorax and abdomen before extending to the forelimbs followed by the hind limbs. These changes were not found in the head region.

3.4.2.2 Early decomposition stage

As the carcasses entered the early decomposition stage, a green discolouration was apparent on the remains, first beginning at the lower right quadrant of the abdomen before extending to other regions of the trunk and finally progressing to the limbs and then the head. This is similar to the Brown and Peckmann [14] study where it was found that discolouration first began on the abdominal and neck regions before progressing to the limbs. The pattern of the green discolouration observed in the present study and the rate at which the discolouration occurred was consistent between the two winter trials.

Bloat occurred by Day 10 on all regions of the body including the head and limbs for both trials. It has been suggested by Megyesi et al. [1] that the limbs do not bloat during the decomposition process, however, both winter trials of the current study and a winter 2013 decomposition study conducted in the Hawkesbury [26], found that the limbs do bloat. While the Megyesi et al. [1] study was an international study that suggested bloating to the limbs does not occur during decomposition and this study used a human model rather than a porcine model, it should be investigated further whether it is the local environment of Greater Western Sydney that is influencing the bloating process or if it is the porcine model that influences the pattern of bloating.

Bone exposure of the mandible and maxilla occurred first, followed by bone exposure of the fore limbs and hind limbs. This was consistent across all sixteen carcasses in the two winter trials and is comparable to the 2013 study completed in the Hawkesbury region [26]. It is likely that the bones of the jaw (mandible and

maxilla) and the bones of the legs are exposed first as the musculature in these areas is smaller when compared to the structures overlying the bones of the other body regions. Because there is less soft tissue in these areas and they decompose quicker, exposing the underlying skeletal structures [83]. This may also be due to the facial orifices providing ideal access to food sources and warmth for insects.

In both winter trials, maggots were first observed around the orifices of the face such as the mouth, snout and eyes. Following their appearance at the orifices of the face, maggots were then observed at the junction of the legs and trunk as well as the rear. Each of these areas provides easy access for the maggots to enter the body to feed as they prefer a warm environment characteristic of the internal confines of a body or carcass [65].

As the carcasses neared the end of the early decomposition stage, they developed a black discolouration to the soft tissues as they decayed. This black discolouration was first observed on the tissues of the face, followed by the legs then the trunk in the present study. This is dissimilar to Brown and Peckmann [14] where they first observed the black discolouration on the forelimbs. Perhaps this pattern of discolouration occurs as the smaller musculature of the face and legs in pig carcasses will dry quicker than that of the trunk.

3.4.2.3   Advanced decomposition stage

Once the remains had entered the advanced decomposition stage, the rate at which the changes occurred varied between the trials and individual carcasses. Although the rate was different, the major soft tissue changes observed between these trials were comparable. Initially, in the advanced decomposition stage, all remains began drying as active soft tissue decay slowed dramatically or ceased. The tissues became leathery before the outer shell of the carcass hardened and the remains mummified or desiccated. The bones that were exposed throughout the trials continued to undergo subtle taphonomic changes to the exterior surface such as bleaching from an initial yellow appearance.

Regardless of the trial, all remains only reached the advanced decomposition stage and progressed no further into the skeletonisation stage during winter. This finding is comparable to Bunch's 2009 [111] investigation on the impact of cold climates on the decomposition process of pig carcasses. It was found that after one year, remains

still presented in the advanced decomposition stage and in a similar state to what they were the year prior [111]. This phenomenon was also found in the current investigation.

As will be demonstrated in Chapter 4, the carcasses in the summer trials continued though the advanced decomposition stage to completely skeletonise and enter the extreme decomposition stage. Mummification can occur when conditions are dry and cool [16] conditions that are typical of an Australian winter [102]. As such, this may have provided the ideal setting for the mummification process observed in the winter trials. Three sets of remains mummified earlier during Trial 1 and Trial 3, and all remains that mummified early were located in the shaded microclimate.

3.4.3 Explanation of findings

All sixteen carcasses used in the two winter trials were located in the same location (Bush Block, Western Sydney University) and experienced similar rainfall levels (125.8mm in 2014, and 115.6mm in 2015), wind speed (average 37km/h in 2014 and 32 km/h 2015) and altitude (19m; 62ft), with the greatest difference in the environment being the two microclimates in which they were located.

Through statistical analysis (paired samples t-test) of the average daily temperatures reported by the data loggers in the two microclimates, it was determined that statistically significant differences existed between the two microclimates. In Trial 1 (winter), the temperatures reported by the data logger positioned in the sun was on average 4.62°C higher than the temperatures reported by the data logger in the shade ($p<0.000$). Although the carcasses decomposed differently between the microclimates and statistically significant differences were found between the reported temperatures, ambient temperature did not have a significant effect on the decomposition process of the pig remains. The individual carcasses in Trial 1 (winter) also decomposed differently from one another, suggesting other factors may have affected the decomposition process. Perhaps the carcasses in the shaded microclimate decomposed marginally slower than the sun exposed remains as the canopy of the trees protected them from the elements.

When the paired samples t-test was performed to examine the temperatures reported by the two data loggers in Trial 3 (winter), it was found there was, on average, only a 0.5°C difference between the two microclimates. This was statistically significant

($p<0.000$) when analysing the daily temperatures across the course of the trial. Given that the remains all decomposed in a similar fashion, additional factors must have influenced the decay process. Possible factors may include the diet and conditions of the pigs prior to being slaughtered, which could influence the presence of the number and type of bacteria in the body [9].

Chapter Four

4 Results- Morphological Changes of the Summer Trials

4.1 Introduction

This chapter explores the gross morphological changes observed on decomposing pig remains over two summer periods: Trial 2 (2014/15) and Trial 4 (2015/16). Each trial was conducted over a 12 week period, from December 9th 2014 to March 9th 2015 (Trial 2 summer) and December 8th 2015 to March 8th 2016 (Trial 4 summer). Each trial observed the decomposition process on eight pig carcasses, with a total of sixteen carcasses used across the two summer trials. During each trial, four pig carcasses were placed in a shaded environment and four carcasses were placed in a sun exposed environment, to assess the effect of different microclimates on the decomposition process. Descriptions of the morphological changes commonly observed over the 12 week trials were recoded according to standard decomposition stages published by Megyesi et al. [1] and Galloway et al. [16], as described in Chapter 1. Table 4-1 is a comparison of the average time taken for remains from both summer trials to commence each stage of decomposition as described by Galloway et al. [16] and Megyesi et al. [1]. Time taken is measured in days since known date of death (Day 0).

Table 4-1 Comparison of the summer trials when remains first entered the decomposition stages, as described by Megyesi et al. [1] and Galloway et al. [16].

Decomposition stage	Trial 2 (summer) 2014/15	Trial 4 (summer) 2015/16
Fresh	Day 0	Day 0
Early decomposition	Day 2	Day 2
Advanced decomposition	Day 9	Day 15
Skeletonisation	Day 27	Day 27
End of Trial	Day 90	Day 90

4.2 Trial 2 (summer, 2014/15)

Figure 4-1 summarises the major soft tissue changes observed during Trial 2 (summer 2014/15). Progression of decomposition from fresh (Figure 4-1a), through to bloat (Figure 4-1b), and advanced decomposition stages (Figure 4-1c-d) was rapid (Table 4.1) and all remains reached the skeletonisation (Figure 4-1e-f) stage. Details of the specific observable changes within each decomposition stage are presented below.

Figure 4-1 Representative photographs of pig carcasses taken during Trial 2 (summer) 2014/15 as they progressed through the following decomposition stages a) fresh; no visible changes b) early decomposition; characterised by full bloat, colour change, maggot masses c) early decomposition; post-bloat, sinking of the body, loss of biomass revealing underlying skeletal structure d) advanced decomposition; extensive bone exposure and loss of soft tissues e) skeletonisation; more than 50% bones exposed, some dried skin and connective tissues remaining; bones undergoing bleaching f) complete skeletonisation.

4.2.1 The Fresh Stage

The fresh stage began on the day of slaughter (Day 0) for Trial 2 (summer) and continued over the following two days, concluding on Day 3 (Figure 4-1a). During this fresh stage, the presence of flies was observed and oviposition had occurred predominantly around the facial orifices on Day 3. Remains presented with minor lividity on the neck, however, no further changes were observed until Day 3 when the early decomposition stage commenced (Figure 4-2).

Figure 4-2 Representative sun exposed carcass in the fresh stage in Trial 2, as described by Galloway et al., where no changes to the tissues has yet occurred except where lividity is present, as indicated by the red square.

4.2.2 Early Decomposition Stage

The early decomposition stage began on Day 3 for all carcasses in this trial (Figure 4-1b). The beginning of this stage was characterised by the presence of bloat (Figure 4-3) which all carcasses had reached by this time point. Bloat was observed to occur rapidly and in all body regions (head, limbs, and trunk) with all carcasses in a state of full bloat on Day 3. Maggot activity had commenced and was most predominant around the head region where active soft tissue decay was occurring. A golden-brown discolouration on the abdomen and legs was also observed for all carcasses where the skin had slipped.

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Figure 4-3 Representative carcass demonstrating the presence of full bloat on the trunk, limbs and head (not shown) at Day 3.

Day 5 of this trial saw a substantial number of decomposition changes occur. Carcasses positioned in the sun had moved into the post-bloat phase, characterised by deflation of the abdominal region following the release of accumulated gases [48, 61] and displayed blackened facial tissues. The sun exposed carcasses also showed bone exposure of the leg bones and skull, predominantly the facial bones, with one carcass also showing bone exposure of the shoulder (Figure 4-4). Carcasses located in the shade remained in bloat, but still displayed blackened tissues of the face. Skin on the legs also blackened in the shaded carcasses (Figure 4-5). Skin slippage and bone exposure of the upper and lower jaw bones (maxilla and mandible) was only observed at Day 5 in the shaded carcasses. Fly and maggot activity was extensive for all of the carcasses. Large maggot masses featured predominantly at the rear and fore limbs and flies surrounded and covered the body in abundance.

Figure 4-4 Representative carcass in the sun exposed microclimate displaying the post-bloat state showing bone exposure at the shoulder (blue arrow), presence of maggot activity towards the rear (orange arrow), skin slip at the forelimbs (purple arrow).

Figure 4-5 Representative carcass in the shade displaying bloat, blackened facial tissues (blue arrow), skin slip of the right fore limb and thorax (purple arrow), with fly and maggot activity present (orange arrow).

By Day 6, tissues had blackened and sloughed away from the bones of the sun exposed carcasses. Carcasses in the sun demonstrated increased bone exposure of the skull, ribs, legs and vertebral column, with the bones exhibiting a black, greasy exterior. Notably, each of the eight carcasses had patches of flesh where the skin and hair had slipped away and revealed a red-maroon and golden brown colouring that was not observed in the winter trials (Figure 4-6). Facial tissues had begun receding, starting at the mouth and snout region revealing the bones of the upper and lower jaw. The soft tissues of the abdomen appeared shredded due to invasion and consumption of the tissues by maggots. One carcass (shade) began showing signs of accelerated decomposition in comparison to the other carcasses. This carcass had the greatest amount of maggot activity, with maggots concentrated around the rear and abdomen. This caused the rear of the shaded carcass to collapse due to the loss of integrity of the soft tissue resulting in the bony outline of the pelvis and legs (Figure 4-6). This was not observed at this time point in any other carcass.

Figure 4-6 Shaded carcass (Trial 2: summer, 2014/15) showing a sunken rear and abdomen due to the loss of the internal structural integrity of the remains. The blue arrows and inset shows the red-maroon colouring observed in the summer trials only.

Day 7 showed further soft tissue decomposition resulting in greater skeletal exposure, specifically of the ribs and vertebral column. The outer soft tissues were black and wrinkled, with decomposition of the soft tissue mostly occurring on the

legs, back, face and rear. Exposed bones were beginning to lighten and were discoloured a yellow-brown colour, but were beginning to dry (Figure 4-7), with stringy, black connective tissue adhering to the bones. Flesh continued to slough away from the bones for each of the carcasses. Remaining tissues took on a leathery appearance on those remains located in the sun, but for the carcasses deposited in the shade, decomposition of the soft tissue was still very moist. Facial tissues for each of the eight carcasses had sagged making the facial features unrecognisable.

Figure 4-7 Representative carcass in Trial 2 displaying blackened soft tissues and the red arrows highlight areas of bone exposure of the ribs and hind legs.

Decomposition changes were occurring rapidly in Trial 2 and on Day 8 the ribs disarticulated from the vertebral column of two sun exposed carcasses and despite sagging, the facial tissues were soft and brown but noticeably drying. All other remaining tissues of the trunk and legs were black, and active decay persisted. The skin was wrinkled with a dark brown-black discolouration and decomposition fluid purging from the head and neck regions. Tissue discolorations continued to darken for all sets of remains with tissues of the face sagging completely rendering the facial features even more unrecognisable.

4.2.3 Advanced Decomposition Stage

Increased bone exposure and soft tissue decay was present on all sets of remains on Day 9 of Trial 2 and tissues had developed a brown-black discolouration on all remaining tissue (Figure 4-1d). Some maggots remained at this point in the PMI but were considerably reduced in abundance.

The period between Days 9 and 13, showed minimal external soft tissue changes with the exception of substantial biomass loss from each of the carcasses. At this time point, carcasses placed in full sun were substantially decomposed in comparison to carcasses placed in the shade.

The skull, legs, ribs, vertebral column, shoulder and jaw of the sun exposed carcasses were completely exposed and were drying and lightening due to prolonged exposure to the sun causing bleaching.

On Day 19, the upper torso of a shaded carcass from the head to the middle of the trunk (just inferior to the ribs) had near complete skeletonisation, while the abdomen to the hind legs and rear still held flesh. The lower half of this shaded carcass had a black discolouration with a red/brown rear and mummified tissues of the hind legs. The skeletonised upper torso retained a black, greasy substance on the bones with minimal tissue adhering and all of the exposed bones beginning to disarticulate (Figure 4-8). This shaded carcass was thus considered to have entered the skeletonisation stage. All other carcasses placed in the shade had large amounts of soft tissue covering the body with some bone exposure of the leg bones and regions of the skull (Figure 4-9).

Figure 4-8 A shaded carcass at Day 19 showing the upper torso of the body to be skeletonised from the head to the ribs and covered with a greasy black substance.

Figure 4-9 Representative carcass in the shaded microclimate displaying large amounts of soft tissue with some bone exposure of the legs and face. This is in comparison to the shaded carcass above which was half skeletonised at this point in the PMI.

Each of the remaining carcasses in the shade had decomposed in a similar fashion to one another throughout the trial. All shaded carcasses demonstrated a sunken abdomen, bone exposure of the legs and skull, and a brown-black and golden discolouration to remaining tissues. The legs had started to disarticulate, bone exposure of the skull and ribs continued to increase, with the exposed retaining a greasy, black exterior.

Between Day 20 and Day 37 (Trial 2), very few observable changes occurred to the eight carcasses. By this time, the remains were all nearing the end of the advanced decomposition stage and progressing into the skeletonisation stage, with the exception of one sun exposed carcass which continued to be the least decomposed carcass and still retained a significant portion of the outer soft tissues. Minimal changes to the remains occurred until Day 64, with the exception of increased bone exposure and visual changes to the bone surface.

Throughout the advanced decomposition stage, all carcasses displayed characteristics which overlap the advanced and skeletonisation stage. As defined by Galloway et al. [16] and Megyesi et al. [1], remains are classified in the advanced decomposition stage when bone exposure is less than 50% of the total skeleton. As such, carcasses were considered to be in the skeletonisation stage only after Day 64 when they displayed more than 50% bone exposure.

4.2.4 Skeletonisation Stage

Day 65 saw further changes occur to the eight carcasses. 50% of the carcasses in both the sun and shade were now completely skeletonised with extensive bleaching of the bones (Figure 4-1e). The bones of the legs of all carcasses had begun to disarticulate with some connective tissues remaining. The legs, ribs, vertebral column and pelvis of the sun exposed carcasses displayed further bleaching from the sun and appeared white Figure 4-10.

Figure 4-10 Representative sun exposed carcass demonstrating extensive bone exposure and bleaching of the bones of the legs, ribs, vertebral column and skull (as indicated by the red arrows).

By the end of the second month of the PMI, carcasses were approximately 85% skeletonised, with the exception of one shaded carcass that had reached full skeletonisation at this point. All carcasses progressed to the skeletonisation stage by Day 72. All sets of remains were almost totally skeletonised with only minor connective tissues remaining.

4.3   Trial 2 (summer) – Summary of the main findings

Each set of remains in Trial 2 (summer) progressed through to the early decomposition stage quickly and changes to soft tissues occurred rapidly. All eight carcasses progressed to a fully bloated state by Day 3 and bloated to such a degree that the carcasses rolled onto their backs and some carcasses were displaying intestinal exposure through burst abdominal walls. This phenomenon is due to the extensive accumulation of gases within the abdomen. By Day 5, all carcasses had entered a post-bloat state and the soft tissues had turned black. It was on this day that the first sign of bone exposure was observed on the legs and skull.

The exposure of the underlying skeleton progressed quickly and bones of the trunk including the ribs and vertebral column were visible by Day 6. The ribs disarticulated from the vertebral column on approximately Day 8.

Decomposition progressed steadily for the next few days and slowed only once the carcasses were mostly skeletonised. Exposed bones retained a black, greasy exterior and did not show signs of bleaching until approximately Day 55. All remains in Trial 2 (summer) completely skeletonised with only limited connective tissues remaining on the limbs and vertebral column for each carcass at the end of the trial at Day 90.

4.4 Trial 4 (summer, 2015/16)

Figure 4-11 summarises the major soft tissue changes observed during Trial 4 (summer 2015/16). The fresh stage began on the day of slaughter (Day 0) of Trial 4 (summer) (Figure 4-11) and continued over the following two days, concluding on Day 2 of the trial. Progression of decomposition from fresh (Figure 4-11a) through to bloat (Figure 4-11b) and advanced decomposition stages (Figure 4-11c-d) was rapid and all remains entered the skeletonisation stage (Figure 4-11e-f). Detailed descriptions of the major soft tissue changes observed during each decomposition stage are discussed below.

Figure 4-11 Representative photographs of pig carcasses taken during Trial 4 (summer) 2015/16 as they progressed through decomposition a) fresh, no visible changes b) early decomposition; full bloat with fly presence c) advanced decomposition; sagging of the body, loss of biomass, first sign of bone, exposure of visceral organs d) advanced decomposition; extensive bone exposure, bones undergoing bleaching, some connective tissues remain e) remains more than 50% skeletonised with some desiccated skin remaining on the limbs and neck.

4.4.1   The Fresh stage

The fresh stage commenced from the time the pigs were killed on (Day 0) and continued until approximately Day 2 (Figure 4-11a, Figure 4-12). Minimal insect activity (mostly flies) was observed. During this stage, all carcasses demonstrated lividity on the thorax and limbs. Remains had progressed to a fully bloated state by Day 3 of Trial 4 signalling the end of the fresh stage and commencement of the early decomposition stage.

Figure 4-12 Representative carcass (shade) demonstrating the rapid progression from a) the fresh decomposition stage on Day 0 where no observable changes had yet occurred to b) the early decomposition at Day 3 where remains had bloated.

4.4.2   Early Decomposition stage

The early decomposition stage began on Day 3 for all carcasses in this trial, signified by the carcasses presenting with full bloat of the trunk, limbs and face (Figure 4-11b). A small number of carcasses, one from each microclimate, showed the

intestines to have perforated the abdominal wall and were now exposed externally (Figure 4-13).

Figure 4-13 Representative carcass demonstrating full bloat where the intestines perforated the abdominal wall likely due to excessive gas build up, as shown by the arrow.

At Day 4, 24 hours after reaching full bloat; all remains across both microclimates had entered the post-bloat stage. A majority of carcasses, predominantly the sun exposed carcasses, showed bone exposure of the facial skeleton, explicitly the mandible and maxilla, and the fore limbs. Large maggot masses were present on the remains, particularly at the facial orifices, thoracic region closest to the soil and forelimbs. Tissues of the legs, lower abdomen and head had blackened. Active decay was present throughout each of the bodies at the heads and neck, trunk, and limb regions. Each set of remains had skin slippage on the legs, head and rear, revealing a red-maroon/golden discolouration previously observed in Trial 2 (Figure 4-14).

Figure 4-14 Representative pig carcass displaying large maggot masses at the thoracic and fore limbs regions, as well as skin slippage along the length of the trunk and neck. The beginning of the red-maroon/golden discolouration is becoming visible on the left hind leg, as indicated by the orange arrow.

Figure 4-11c demonstrates an example of the soft tissue changes observed on Day 6 for all carcasses. Carcasses had lost substantial amounts of biomass and the trunk of the carcasses had decomposed the most, revealing the bones of the ribs and vertebrae. The soft tissue of the cranial vaults and legs had also decomposed in most places revealing the underlying bones. All exposed bones were covered in a greasy black substance and some black stringy connective tissues remained. The tissues surrounding areas of increased decomposition were black in colour. Two shaded carcasses also had disarticulation of the hind limbs (Figure 4-15). Day 6 marked the beginning of the transitional changes and overlapping of the early and advanced decomposition stages.

Figure 4-15 Representative carcass (shade) demonstrating significant soft tissue decomposition with bone exposure of the ribs, vertebral column and various bones in the neck region (red arrows). This image shows the positioning of the hind legs of Pig 2 after they disarticulated post-bloat (orange arrows) (Trial 4 2015/16).

All carcasses were following a similar decomposition sequence in regards to the pattern of decay at particular body regions and timing. The four carcasses located in the sun exposed microclimate were decomposing at a slightly faster rate to the shaded carcasses, but not considerably.

From Day 8 to Day 14 of Trial 4, minimal changes were observed. Biomass loss as well as degree of bone exposure of the ribs, vertebrae, limbs and skull did not change during this period. A greasy substance continued to cover the bones. On Day 14 and 15 however, 62.2mm of rain fell and the greasy substance covering the bones and decomposition fluids surrounding the carcasses was washed away to reveal clean, dry bones. Figure 4-16 demonstrates the progression of the bones from having a greasy exterior (Figure 4-16a) to a clean and dry exterior (Figure 4-16b) following the period of rainfall on Days 14 and 15.
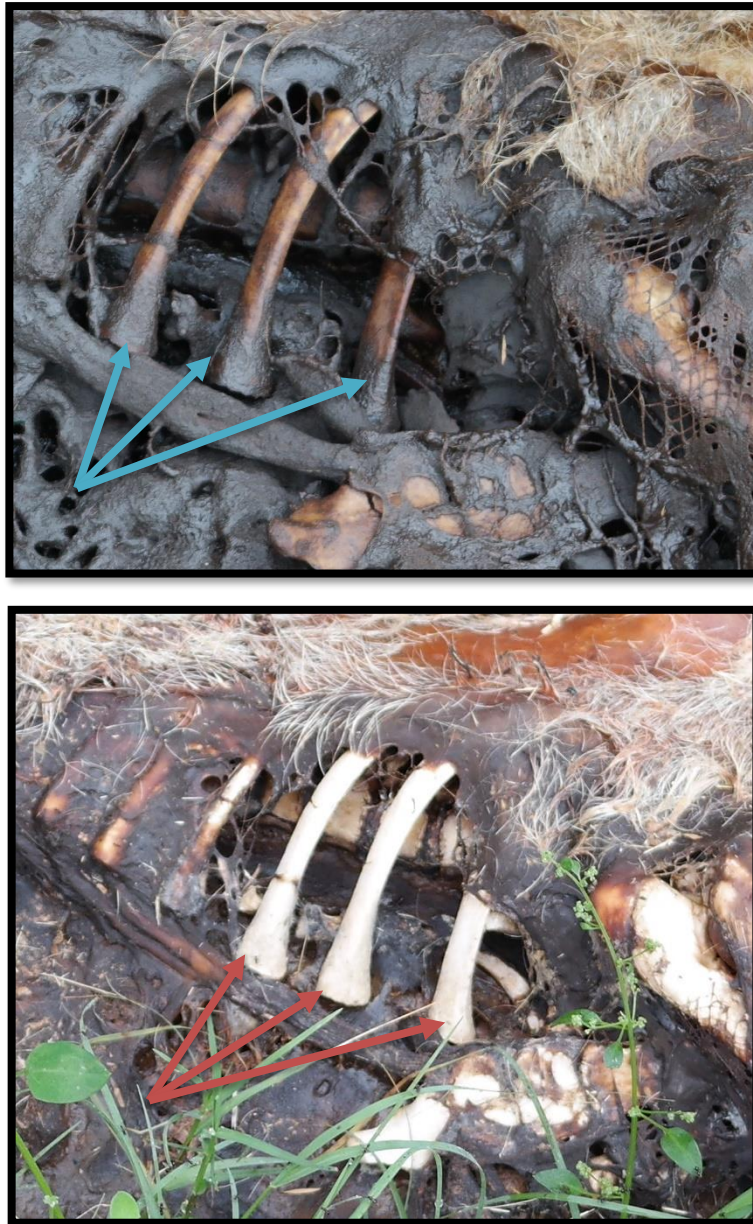
Figure 4-16 a) Greasy substance covering the bones of the ribs (blue arrows) before a period of rainfall. b) after rainfall, revealing clean and drying bones (red arrows).

4.4.3 Advanced decomposition stage

Few new taphonomic changes were observed between Days 15-26 but carcasses continued drying during this time and after the period of rainfall. From Day 27 to Day 30 in Trial 4, 157.8mm of rain fell in the area and the remains paled in colour and appeared to rehydrate (Figure 4-17). Following the conclusion of this rainy period on Day 30, remains in the shaded environment demonstrated extensive bone

exposure and very little soft tissue remained on the middle of the trunk, right hind leg and left forelimb.

The sun exposed carcasses all demonstrated a similar decomposition pattern and sequence. These four carcasses were approximately 50% skeletonised but were still considered to be in the advanced decomposition stage. According to Megyesi et al. [1] and Galloway et al. [16], a body is classified as in the skeletonisation stage when it is more than 50% skeletonised. Each carcass had extensive bone exposure at the skull, neck, legs, vertebral column and ribs and some "stringy", pale flesh remained at the lower abdomen, rear and under the jaw. The bones exposed in these carcasses were visually different to those exposed bones of the shaded carcasses. The bones of the carcasses in the shade maintained a golden brown exterior, whereas those bones located in the sun were clean, pale and maintained a white colouring throughout each area of the bones (Figure 4-18). The whiter appearance of the bones of the sun exposed carcasses may be attributed to the rain washing the excess greasy substance such as decomposition fluid from the bones and/or bleaching as a result of their exposure to the sun.

Figure 4-17 Representative carcass (shade) after a period of rainfall. The remaining soft tissues paled in colour and appeared rehydrated.

Figure 4-18 Representative carcass demonstrating the difference in the colouring of exposed bones (red arrows) in a) a shaded carcass and b) a sun exposed carcass.

4.4.4 Skeletonisation stage

Few obvious, observable changes were noticed overall to the remains between Day 27, when the remains skeletonised, and Day 37. At Day 37, the bones of the shaded carcasses were becoming visibly lighter in colour and were beginning to dry out (Figure 4-11e and Figure 4-19). The remaining skin on each of the carcasses, and the bones of the sun exposed carcasses had also begun to dry since the period of rain experienced in the weeks prior.

Rain continued to fall between Day 38 to Day 55 (135.5mm in total) rehydrating the remaining soft tissue. By Day 64 of Trial 4, remains in both microclimates had entered a period of stasis in the decomposition of soft tissue. All carcasses exhibited clean, dry, bleached bones with only minimal soft tissues such as skin and some connective tissues adhering to the bones of the back and shoulder regions. Three sun exposed carcasses had grassy overgrowth on and around the carcasses, whereas one of the sun exposed carcasses, in the same area as these carcasses, did not have any overgrowth of vegetation. The immediate surrounds of this carcass was absent from vegetation creating a silhouette around the carcass, like a cadaveric island had formed without the presence of decomposition fluid and liquefaction commonly seen with a cadaveric decomposition island, however, it is possible the decomposition fluids leeched into the soil.

Figure 4-19 Bone exposure of the ribs, legs and feet of a carcass showing early signs of bleaching of the bones due to exposure to the sun.

Carcasses entered the skeletonisation stage, when they were more than 50% skeletonised [1, 16], at approximately Day 27 and remained in this stage for the length of the trial, concluding on the Day 90. All carcasses rapidly decomposed early in the study causing them to enter the skeletonisation stage early. As skeletonisation was reached early, the remains showed very few changes from the time skeletonisation occurred through to the end of the trial.

4.5   Trial 4 (summer) – Summary of the main findings

Remains in Trial 4 (summer) also progressed through to the early decomposition stage rapidly, and, similar to the remains in Trial 2 (Summer), had entered a full bloat state by Day 3 and rolled onto their backs due to the extensive accumulation of gases with the abdomen.

Perforation of the abdominal wall with protruding intestines was observed around Day 3. This phenomenon was only observed in the summer trials, with no visceral organ exposure during either of the winter trials. This is likely due to the extensive and rapid accumulation of gases within the abdomen of the summer carcasses, hence the high degree of bloat that was not observed in the winter trials.

All eight carcasses entered a post-bloat state by Day 4 and the sun exposed carcasses showed the first signs of bone exposure at the head and forelimbs, signalling an overlap of the early and advanced decomposition stages. Tissues had begun to blacken, particularly at the mouth and limbs. Carcasses in the summer trials did not demonstrate the typical green discolouration observed on the abdomen early in the early decomposition stage, but instead demonstrated a black-dark green discolouration, usually observed toward the end of the early stage. However, it is possible that this colour change was missed because the remains progressed so quickly through these trials.

The carcasses in Trial 4 (summer) all followed a similar decomposition rate and pattern. Throughout this trial a substantial amount of rainfall fell causing the remains to rehydrate and initiate active decay. Each time rain fell, the remains were washed of any liquefied tissue and the bones were cleaned of the greasy substance that adhered to them.

4.6 Discussion

4.6.1 Differences between the two summer trials

The carcasses in both trials decomposed at a similar rate until they reached the advanced decomposition stage. At this time, the eight carcasses in Trial 2 entered the advanced decomposition stage at Day 9 whereas it was not until Day 15 when the eight remains in Trial 4 reached this stage. In Trial 2, the remains located in the sun were the first remains to show bone exposure of the skull and limbs, whereas in Trial 4 it was the remains that were located in the shade which first demonstrated exposure of these bones. Notably, the carcasses in the individual summer trials entered the advanced decomposition stage at different times, however, commenced the skeletonisation stage at the same time i.e. Day 27.

4.6.2 Similarities between the two summer trials

4.6.2.1 Fresh stage

In both trials, the sixteen carcasses remained in the fresh stage for two days only. A heavy presence of flies was observed on and surrounding the body and ovipositing around the orifices of the face, for all remains. During the fresh stage, each carcass had lividity particularly on the thorax and limbs. The abundance of flies and

formation of lividity occurred at similar times during this period by approximately Day 2, a result similar to the Brown and Peckmann [14] study where flies were also observed on Day 2.

4.6.2.2 Early decomposition stage

Upon entering the early decomposition stage, remains demonstrated full bloat and a black-green discolouration to the abdomen, thorax and limbs. This was different to the characteristic green discolouration that is reported in the literature which is suggested to signify the commencement of the early decomposition stage and was observed in the winter trials [1, 16, 58, 61, 87]. All carcasses in the two summer trials bloated within three days of the commencement of the trials, resulting in the carcasses rolling onto their backs from the lateral position. This may be caused by the extensive and rapid accumulation of gases within the body [58]; a phenomenon that was not observed in the winter trials where remains in those trials only partially bloated. This rapid onset of bloating in conjunction with a black-green discolouration is comparable to Enwere [112], Larizza [13] and Parsons [53] summer studies where bloating of carcasses was observed on Day 2 of each of these studies. To note, all regions of the body (head and neck, trunk, limbs) all bloated in the summer trials, this pattern is comparable to what was observed in both winter trials of the current study.

As quickly as the sixteen carcasses in the summer trials bloated, they all quickly entered the post-bloat phase by Day 4 and 5. Ayers [37] and Parsons [53] also observed post-bloating to occur on Day 4 of their decomposition study. This resulted in the carcasses beginning to sag predominantly in the trunk, large maggot masses formed around the head region and lower abdomen, and the first signs of bone exposure were observed most notably at the maxilla, mandible and forelimbs. It has been postulated that exposure of these particular bones occurs first as the musculature within the areas of the jaw and limbs is much smaller than other body regions [113, 114]. It should be noted that it was during the bloat phase that exposure of the bones was first observed in both summer trials. Soft tissue decomposition progressed rapidly in these trials and by Day 7, bone exposure was extensive, revealing a black greasy exterior on the bones.

In both summer trials, a red-maroon discolouration was observed on the remains once the hair and layers of dermis began to slip, as seen previously in Figure 4-6, which again, was not observed in the winter trials.

While the rain that fell in the 2015/16 summer trial rehydrated the dried tissues of the remains, the reactivated decay process soon slowed again before the remains entered into the advanced decomposition stage. The rainfall that occurred during Trial 2 also rehydrated the remains in this trial before decomposition once again slowed.

4.6.2.3 Advanced decomposition stage

Upon entering the advanced decomposition stage, each carcass was losing substantial biomass causing the remains to cave in dramatically, revealing the underlying bone structure. Similar patterns of drying were observed between the individual carcasses and greater bone exposure was occurring particularly along the vertebral column and ribs for all carcasses.

The morphological changes observed during the early and advanced decomposition stages in Trial 2 and Trial 4 occurred rapidly. However, it was observed at this time point in the PMI that those carcasses positioned in the sun were decaying marginally, but not measurably, faster in both summer trials, a result found previously in the literature [14, 110].

While the carcasses in the two individual trials entered the advanced decomposition stage at slightly different rates, they equally remained in this stage until Day 27 before they progressed to the skeletonisation stage.

4.6.2.4 Skeletonisation stage

By Day 27, all 16 sets of remains in the summer trials had reached an early skeletonisation stage, where less than half of the body was skeletonised (as described by Galloway et al. [16]). This result is comparable with to Leblanc and Strongman's [115] where it was found remains reached the skeletonisation stage by Day 26 of their trial. Mann et al.'s [9] reported skeletonisation could occur within two-four weeks in a Tennessee environment during the summer months. Komar [54] also found skeletonisation was reached rapidly in the summer in Edmonton, Alberta and reported remains skeletonising within 2-4 weeks.

Once the carcasses entered the skeletonisation stage in the present study, further bone exposure to the bones of the trunk, pelvis and head were documented and the

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exposed bones gradually underwent the bleaching process. Rain fell during the skeletonisation stage in both summer trials washing the greasy exterior from the bones, subsequently cleaning them, which promoted the drying of the bones.

4.6.3 Explanation of the findings

The 16 carcasses used in the two summer trials progressed through the fresh, early and advanced decomposition stages at very similar rates. These carcasses exhibited similar morphological changes and the rate of decay was comparable among each set of remains. This was different to the pattern observed in the two winter trials where the individual carcasses decomposed differently from one another. Perhaps as the soft tissue decomposition process was so rapid in the summer trials it eliminated the variability that was documented in the winter trials. The quick progression of decay was a result of the warm, humid conditions characteristic of temperate Australian summers [102].

Both summer trials experienced similar climatic averages. These included rainfall levels (335mm in 2014/15 and 387mm in 2015/16), wind speed (39 km/h in 2014/15 and 37 km/h in 2015/16) and the unique environmental features of the research site including the vegetation and soil surface the remains were placed on, remained consistent amongst the trials.

To assess for differences in the decomposition process when remains are located in a shaded or sun exposed microclimate, the shaded carcasses of both Trial 2 (summer) and Trial 4 (summer) were located on a soil surface under the canopy of trees. The sun exposed carcasses of both trials were deposited on the soil surface, in an area where they were exposed to full sunlight for the majority of the day. Through statistical analysis (paired t-test) of the average daily temperatures reported by the two data loggers at the research site, it was determined that statistically significant differences existed between the sun exposed and shaded microclimates in both summer trials ($p<0.000$). The results of the t-test showed the data logger in the sun reported temperatures 2.15ºC higher than those reported by the data logger in the shade in Trial 4, but temperatures in the sun were reported to be only 0.8 ºC higher than the temperatures the data logger in the shade was reporting in Trial 2. Although the remains were deposited in different microclimates and the temperatures recorded by the data loggers in these microclimates were statistically significant, ambient

temperature was not a statistically significant variable affecting the decomposition process under the current experimental conditions (Chapter 7).

The carcasses in both summer trials decomposed at similar rates and demonstrated a similar decomposition pattern; however, the remains that were deposited in the sun exposed microclimate were visually decomposing slightly faster than their shaded cohort. One possible explanation for this finding may be the result of solar radiation [53]. Solar radiation may affect the temperatures the carcasses were exposed to especially when they are left unprotected to the sunlight and it may have a direct effect on the amount of heat absorbed by the carcass [116].

Chapter Five

5 Results- Testing the Published Methods (The Winter Trials)

5.1 Introduction

The following section presents the results of the validation of the currently published methods [1-3] for estimations of PMI for Trial 1 (winter 2014) and Trial 3 (winter 2015), of the present study. This section will demonstrate:

- ❖ the differences in the recorded climatic data will be analysed to determine if there is a statistically significant difference between the three recording devices. This will determine if there is a difference between weather data recorded at the site and data recorded by the local weather station. If a difference exists, the methods will be evaluated using all temperature recording devices to determine if this impacts on the accuracy of the methods
- ❖ the relationship between
  - o (i) the PMI and the TBS (as a representation of decomposition),
  - o (ii) the PMI and the percentage of soft tissue decomposition that has occurred,
- ❖ the relationship between the predicted values of ADD and PMI generated by the published protocols, namely
  - o Megyesi et al. [1] (Equation 1),
  - o Marhoff et al. [2] (Equation 2), and
  - o Vass [3] (Equation 3)

and the known PMI and ADD of the remains in the current study.

- ❖ the validity of the Megyesi et al. [1] protocol, the Marhoff et al. [2] formula and the Vass [3] PMI formula in Trial 1 and Trial 3 of the current study will be examined to determine if they can be applied accurately in a Western Sydney region.

5.2 Comparison of the climatic data during the winter trials

As each of the three methods [1-3] to be evaluated in the current study rely on temperature to make a PMI determination, temperature data was recorded using three separate devices. Two of these devices were temperature data loggers positioned at the research site on Western Sydney University's Hawkesbury campus. One of these data loggers was located in an area receiving shade for the majority of the day (data logger 1), while the other data logger was positioned in an area receiving direct sunlight during the day (data logger 2). These devices were attached to the cage of one carcass in each of the microclimates. The third medium used to obtain temperature data was the local weather station for the Bureau of Meteorology (BoM) located at Richmond Royal Australian Air Force (RAAF) base approximately 4.7km from the research site.

5.2.1 Comparison of climatic data: onsite data logger (sun) vs. onsite data logger (shade) for Trial 1 and Trial 3

A paired *t*-test was performed to determine the relationship between the two data loggers at the research site, one positioned in the shade (data logger 1) and the other positioned in the sun (data logger 2). It was revealed that the two devices were reporting different temperatures for their respective microclimates and a statistically significant difference was found between the temperatures recorded by data logger 1 in the shade and data logger 2 in the sun for both winter trials ($p<0.001$).

For data recorded in Trial 1 (winter), it was determined that data logger 2 positioned in the sun was reporting temperatures, on average, 4.62°C higher than the temperatures recorded in the shade. This is important as the two data loggers were positioned only 30 metres apart but recorded significantly different temperatures for the microclimates. For data recorded in Trial 3 (winter), it was determined that data logger 2 positioned in the sun was reporting temperatures, on average, 0.5°C higher than the temperatures recorded in the shade. Figure 5-1 demonstrates the relationship between the average daily temperatures recorded by the data loggers in the two microclimates of Trial 1 and Trial 3.
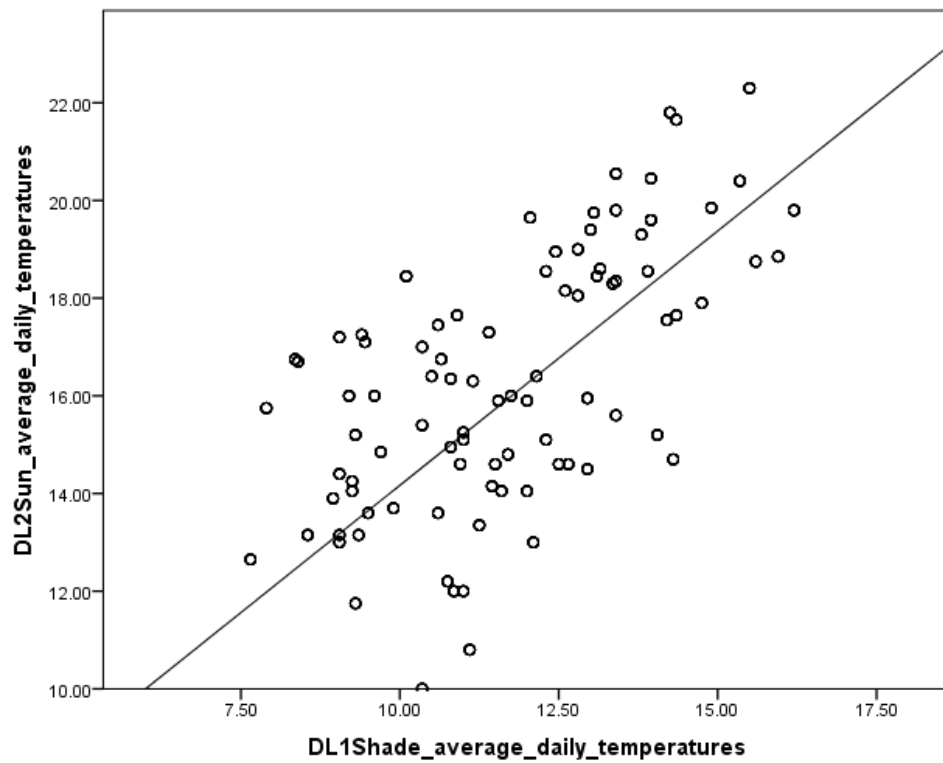
Figure 5-1 Relationship between the average daily temperatures reported by the data loggers at the research site located in the shade (DL1) and in the sun (DL2) in Trial 1. All units are in degrees Celsius (°C).
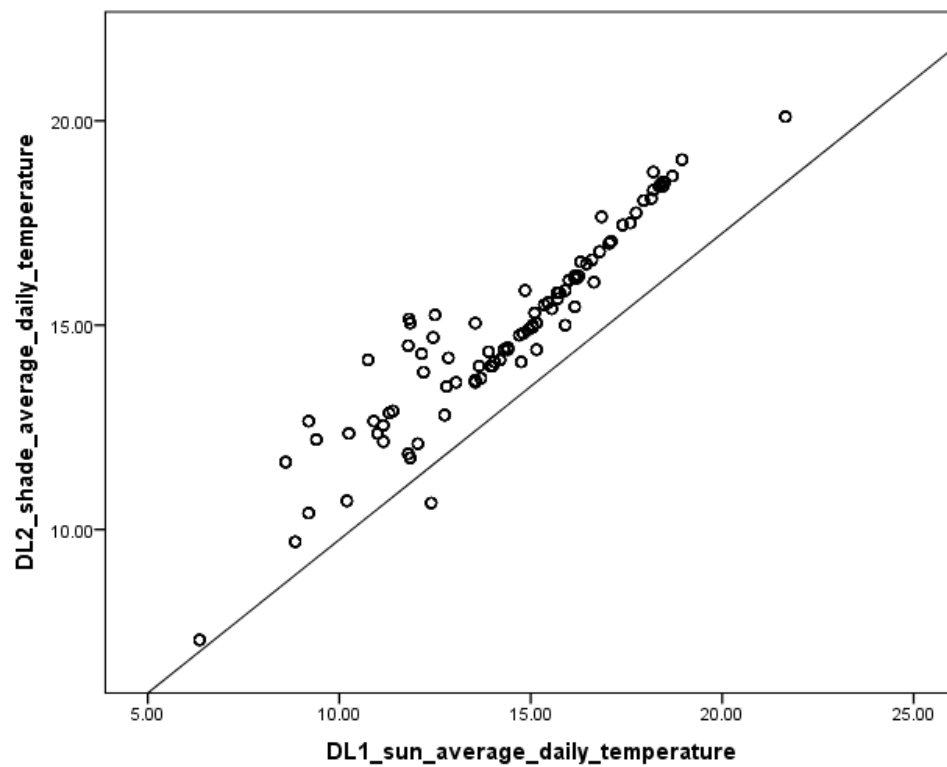
Figure 5-2 Relationship between the average daily temperatures reported by the data loggers at the research site located in the shade (DL1) and in the sun (DL2) in Trial 3. All units are in degrees Celsius (°C).

5.2.2 Comparison of climatic data: BoM weather station vs. onsite data logger (shade)

Figure 5-3 and Figure 5-4 demonstrate the relationship between the temperatures recorded by the BoM weather station and the temperatures recorded by the data logger at the research site positioned in the shade (data logger 1) for Trials 1 and 3. A paired *t*-test showed there was a statistically significant difference between the recordings at the site for both trials and that reported by the BoM weather station (Trial 1; $p<0.001$, Trial 3; $p<0.001$). The data revealed that temperatures recorded by the BoM weather station in Trial 1 were, on average, 0.48°C higher than the temperature reported for data logger 1. For Trial 3, however, data logger 1 at the research site reported temperatures 3.16°C higher than the temperature reported by the BoM weather station.
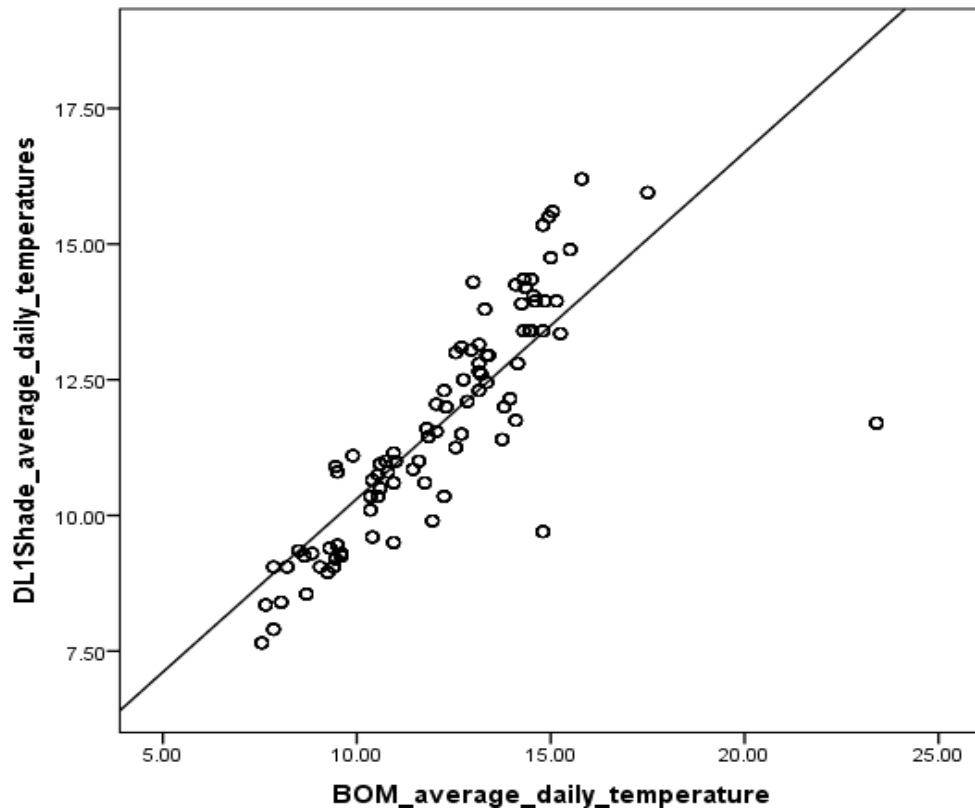
Figure 5-3 Relationship between the average daily temperatures reported by the BoM weather station and the temperatures recorded by the onsite data logger located in the shade (DL1) for Trial 1. All units are in degrees Celsius (°C).
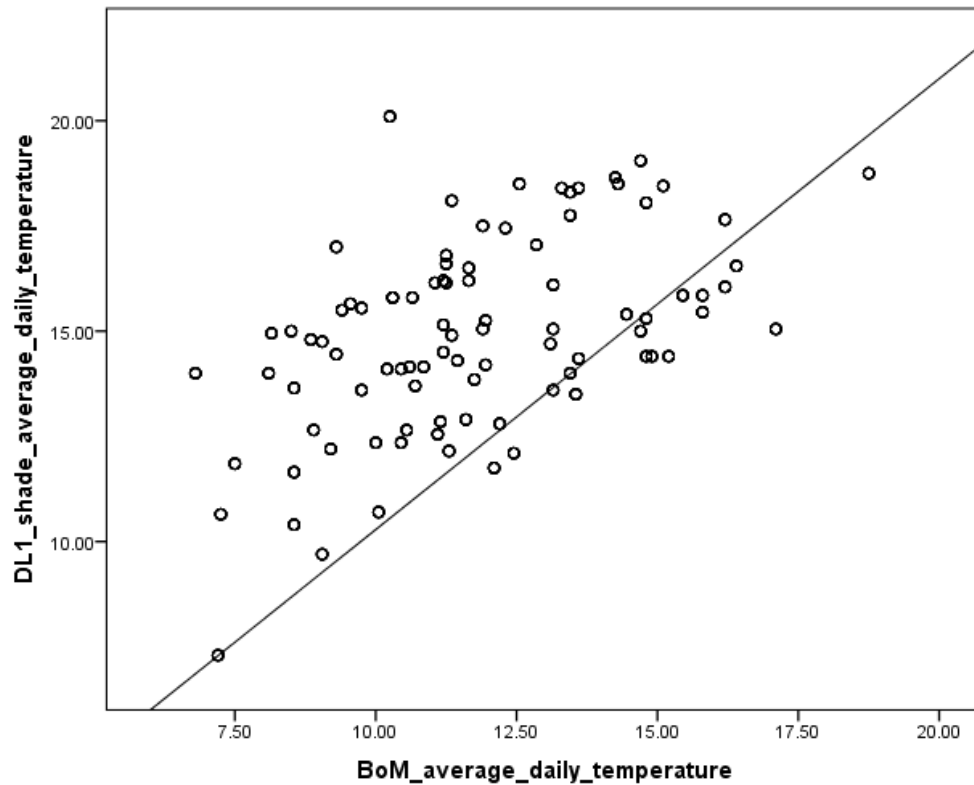
Figure 5-4 Relationship between the average daily temperatures reported by the BoM weather station and the temperatures reported by the onsite data loggers located in the shade (DL1) for Trial 3. All units are in degrees Celsius (°C).

5.2.3 Comparison of climatic data: BoM weather station vs. onsite data logger (sun)

A paired *t*-test was also performed to determine the relationship between the temperatures reported by the BoM weather station at Richmond RAAF base and the temperatures recorded at the research site for the data logger positioned in the sun. There was a statistically significant difference between the temperatures recorded at the research site and the BoM weather station during both 2014 ($p<0.001$) and 2015 ($p<0.001$) winter trials. The temperature data recorded by the data logger in the sun (data logger 2) at the research site was, on average, 4.14°C higher than the temperature reported by the BoM weather station for the area in Trial 1 and 2.65°C higher than temperatures recorded by the BoM weather station for Trial 3. Figure 5-5 and Figure 5-6 shows the relationship between the temperature recorded by the data logger in the sun (data logger 2) and that of the BoM weather station for the two winter trials.
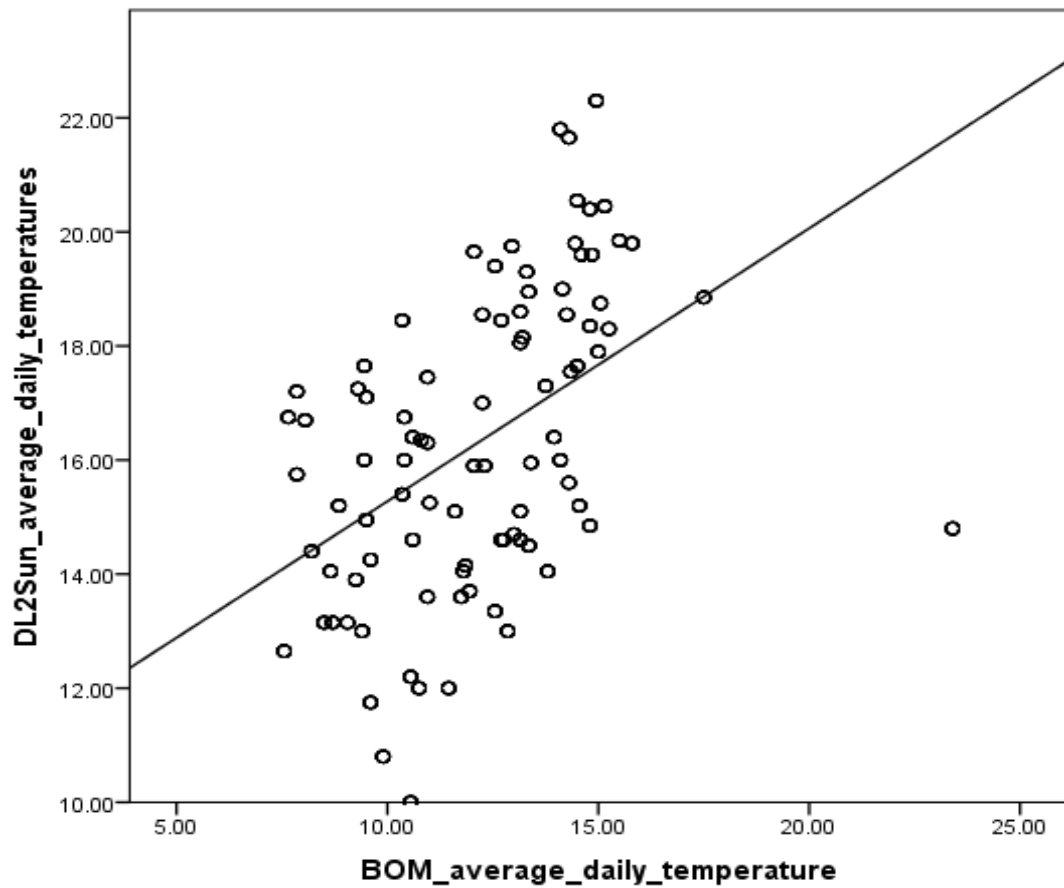
Figure 5-5 Relationship between the average daily temperatures reported by the Bureau of Meteorology (BoM) and the temperatures reported by the onsite data loggers located in the sun (DL2) for Trial 1. All units are in degrees Celsius (°C).
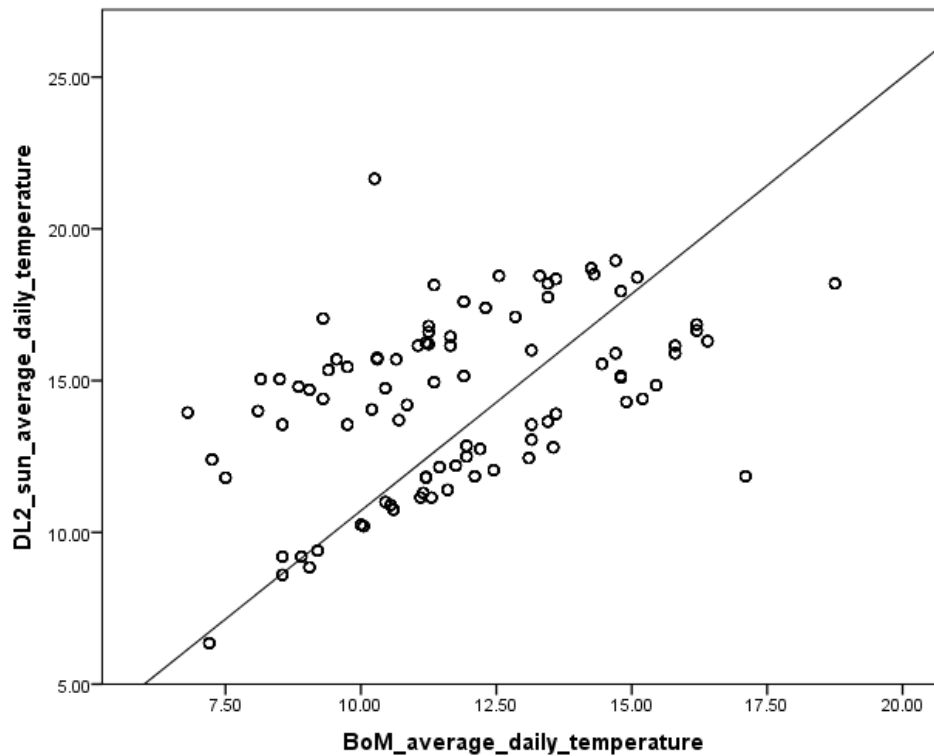
Figure 5-6 Relationship between the average daily temperatures reported by the Bureau of Meteorology (BoM) and the temperatures recorded by the onsite data logger located in the sun (DL2) for Trial 3. All units are in degrees Celsius (°C).

5.2.4 Discussion

Statistically significant differences were found when evaluating the relationships between the three temperature apparatus. As such, it was concluded that the analyses to follow would be performed using the temperature data from all three sources. The temperatures recorded by the two respective data loggers (data logger 1 and data logger 2) would subsequently be used as the temperature variable in PMI calculations of the remains in the individual microclimates (sun and shade). That is, temperatures recorded by data logger 1 would be used when estimating the PMI of the carcasses positioned in the shade and temperatures recorded by data logger 2 would be used in all analyses of the sun exposed remains. The data recorded by the BoM weather station at Richmond RAAF base would also be used to conduct a separate analysis to determine if the Megyesi et al. [1] protocol, the Marhoff et al. [2] formula and the Vass formula [3] could better estimate the PMI using the local weather station data, as this data is likely to be the only source of weather data available in a forensic case or if the methods better estimate the PMI when data is recorded at the scene.

5.3 Validating the published methods for PMI estimates

To determine if the published methods were accurate in a temperate Australian environment, the estimated ADD and PMI using the published equations were plotted against the known ADD and PMI for Trial 1 (Figure 5-8, Figure 5-9, Figure 5-13, Figure 5-14, Figure 5-18) and Trial 3 (Figure 5-11,

Figure 5-12, Figure 5-15, Figure 5-16, Figure 5-20). Visually, for each set of remains and for each protocol tested, there were strong departures from the x=y line, therefore, a linear relationship did not exist between the predicted and known values.

Linear mixed modelling was performed to examine the relationship between the known ADD and PMI of the study and the estimated ADD and PMI found using the predictive equations by Megyesi et al. [1], Marhoff et al. [2] and Vass [3]. The data was computed without the intercept, using the syntax function in SPSS, to determine if the intercept was equal to 1. If the intercept was equal to 1 there was a linear trend between the two variables [pers. comm. Paul Fahey, Western Sydney University].

Each of the linear mixed models showed the PMI/ADD values generated by the predictive methods to be highly statistically different ($p<0.001$) from the known ADD and PMI values in the present study. This means there was a difference between the PMI/ADD that the methods were estimating and the known values of PMI/ADD, which suggests that these equations are ineffective for estimating time since death under the current experimental conditions.

To confirm the result of the linear mixed models, linear regression was performed. If an adjusted r^2 value of 0.80 or higher was achieved (80% accuracy), the published methods were considered to be reasonable predictors of the true ADD or PMI. An adjusted r^2 value of 0.80 was used in order to be comparable with previous studies in the literature [1, 117].

The results of the analyses for the Megyesi et al. method [1], the Marhoff et al. [2] equation for estimating ADD, and the Vass Universal PMI formula [3] are presented individually for Trial 1 and Trial 3 (winter) below.

5.4   Validating the Megyesi et al. ADD method- Trial 1

5.4.1   Trial 1 (winter) body scored using TBS

Figure 5-7 depicts the relationship between the TBS assigned using the Megyesi et al. [1] scoring protocol for all eight carcasses of Trial 1 of the current study against the known PMI.

An exponential growth pattern is initially observed in Figure 5-7 between Day 2 and Day 45. As decomposition was actively occurring during this time, the TBS values assigned to remains were increasing on each sampling day as gross morphological changes were observed on the remains. At approximately Day 45, few visible changes to the remains were detected (see Chapter 3). As decomposition changes were not observed, a new TBS value could not be assigned to the carcass which resulted in the lengthy plateau observed from Day 45 (as indicated by the red arrow) to the end of the trial at Day 90. Observations of each carcass, however, continued to Day 180, despite the primary trial ending at Day 90, to determine the long term stasis of decomposition changes on PMI calculations.

This suggests that the use of TBS as a method of quantifying decomposition for the purpose of PMI calculations may only be effective during the active stages of the decomposition process. To test this, the TBS calculated during the study was used in the Megyesi et al. [1] equation to determine the accuracy of the method in the current experimental conditions.
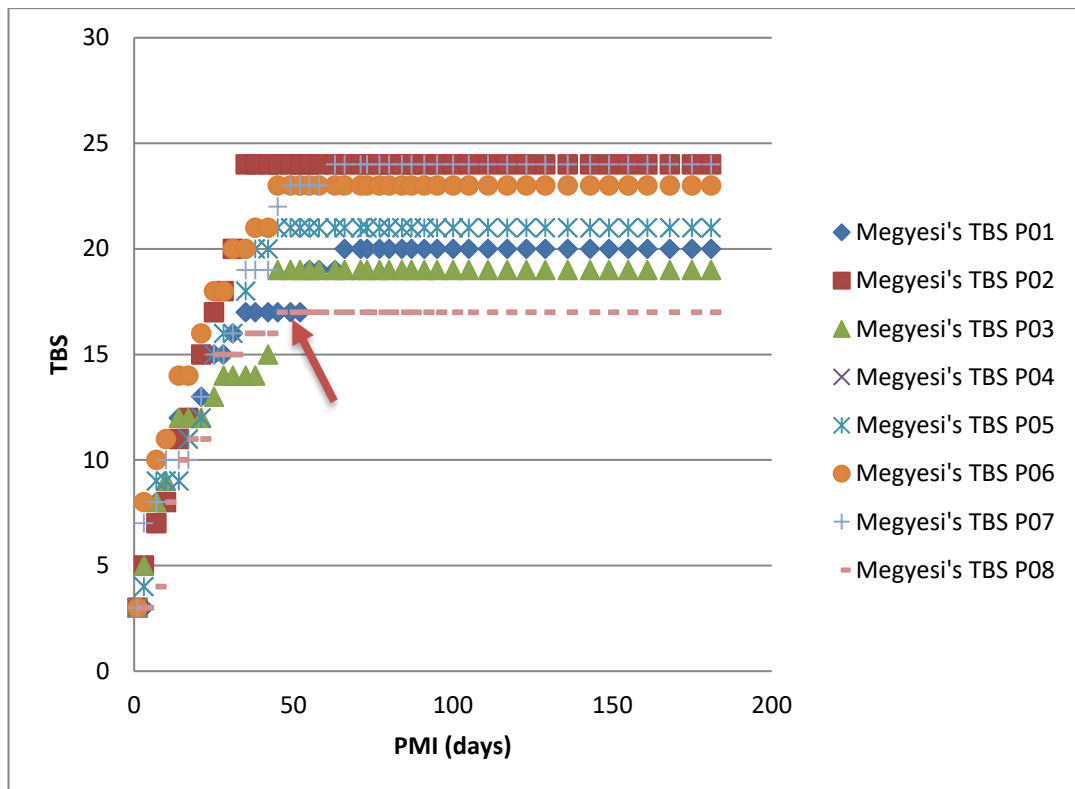
Figure 5-7 Scatterplot of TBS vs. PMI (in days) for all carcasses (Pigs 1-8) to demonstrate the relationship between transpired time and the decomposition of remains for Trial 1. Red arrow indicates the beginning of an extensive plateau where the TBS did not change from Day 45 through to the end of the trial at Day 90.

5.4.2 Using temperature data collected at the research site

Although Figure 5-7 (above) demonstrates that a similar pattern of decay was observed for all carcasses contrary to the microclimate they were located in, for ease of reporting the results the graphs were separated into the shaded and sun exposed microclimates.

Figure 5-8 depicts the predicted ADD against the known ADD of remains, as recorded by the onsite data loggers, located in the shaded (Figure 5-8a) and sun exposed (Figure 5-8b) microclimates. Both microclimates exhibit similarities in predicted and known ADD until approximately 100ADD, after which, separation between carcasses and the two variables is observed.

Due to software limitations, the line of best fit for both the shaded remains and the sun exposed remains appears on both Figure 5-8a and Figure 5-8b. Therefore, the line of best fit for shaded remains is indicated by the purple arrow and the line of best fit for sun exposed remains is indicated by the orange arrow.

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5.4.2.1 Shaded microclimate

The predicted ADD values found using the Megyesi et al. [1] formula for carcasses in the shaded microclimate were compared to the known ADD recorded by the data logger in the shade at the research site (Figure 5-8a).

The results of the linear mixed modelling showed there was a statistically significant difference between the predicted ADD and the actual ADD recorded during Trial 1 when using the Megyesi et al. [1] predictive method on shaded remains. The fixed effect for shaded remains, expressed as the slope, decreased from the intercept (1.54; as recorded for sun exposed remains) by 0.454, resulting in a fixed effect of 1.09 (t (193.5) =-3.32, $p<0.001$). These results suggest that, on average, the formula underestimated the true ADD during Trial 1 and the known ADD of the shaded remains found in this region was 1.09 times greater than the ADD predicted by the Megyesi et al. [1] formula.

Linear regression analysis was performed to determine how well the predicted model was estimating the ADD of the remains in the present study. Regression analysis yielded an r^2 value of 0.388, suggesting the method was a poor predictor of the true ADD of shaded remains in the current study (approximately 40% accuracy) when using temperatures obtained from the 'discovery' site.

5.4.2.2 Sun exposed microclimate

The predicted ADD values found using the Megyesi et al. [1] formula for carcasses in the sun exposed microclimate were compared to the known ADD recorded by the data logger in the sun at the research site Figure 5-8b, using linear mixed modelling.

The results of the linear mixed modelling showed there was a statistically significant difference between the predicted ADD and the actual ADD recorded during Trial 1 when using the Megyesi et al. [1] predictive method on sun exposed remains, with the fixed effect being 1.54 (t (196.3) =16.3, $p< 0.001$). This suggests that, on average, the formula underestimated the true ADD during Trial 1. Hence, the true ADD of the sun exposed remains found in the Hawkesbury region was actually 1.54 times greater than the ADD predicted by the Megyesi et al. [1] formula.

Linear regression analysis was performed to determine how well the predicted model was estimating the ADD of the remains in the present study. Regression analysis yielded an r^2 value of 0.444, suggesting the method was a poor predictor of the true

ADD of sun exposed remains in the current study (approximately 44% accuracy) when using temperatures obtained from the 'discovery' site.

Figure 5-8 The Megyesi et al. [1] prediction of ADD vs. the actual ADD of all carcasses (n=8), where 1.06*x+124 is the line of best fit for carcasses located in the shade and 1.5*x+172 is the line of best fit for the carcasses located in the sun and for Trial 1 winter 2014. Units in 'days °C'.

5.4.3   Using temperature data collected from the BoM weather station

Figure 5-9 depicts the predicted ADD against the known ADD of remains, as recorded by the local BoM weather station, located in the shaded (Figure 5-9a) and sun exposed (Figure 5-9b) microclimates. As observed when using the onsite data loggers, there is a close relationship between known and predicted ADD for all carcasses until approximately 100ADD, after which, a separation between the carcasses and the two variables is clearly observed.

Due to software limitations, the line of best fit for both the shaded remains and the sun exposed remains appears on both Figure 5-9a and Figure 5-9b. Therefore, the line of best fit for shaded remains is indicated by the purple arrow and the line of best fit for sun exposed remains is indicated by the orange arrow.

5.4.3.1 Shaded microclimate

The predicted ADD values found using the Megyesi et al. [1] formula for carcasses in the shaded microclimate were compared to the known ADD recorded by the local BoM weather station.

The results of the linear mixed modelling showed there was no statistically significant difference between the predicted ADD and the known ADD recorded during Trial 1 when using the Megyesi et al. [1] predictive method on shaded remains (t (211.2) =0.083, $p<0.934$). This suggests the use of BoM weather station data with the Megyesi et al. [1] method should improve the accuracy of this method in the current experimental conditions. However, linear mixed model shows that, on average, the formula underestimated the known ADD of the shaded remains during Trial 1 and the known ADD of shaded carcasses in this trial was 1.12 times greater than the predicted ADD when temperatures were recorded at the local BoM weather station.

Linear regression analysis once again determined that the Megyesi et al. [1] method was a poor predictor of ADD of shaded remains when using publicly available temperature data from the nearest weather stations (r^2=0.388).

5.4.3.2 Sun exposed microclimate

The predicted ADD values found using the Megyesi et al. [1] formula for carcasses in the sun exposed microclimate were compared to the known ADD recorded by the local BoM weather station, using linear mixed modelling.

The results of the linear mixed modelling showed there was a statistically significant difference between the predicted ADD and the known ADD recorded during Trial 1 when using the Megyesi et al. [1] predictive method on the sun exposed remains, with the fixed effect being 1.11 (t (210.8) =12.9, $p<0.000$). The results of the linear mixed model suggest that, on average, the formula underestimated the true ADD of the sun exposed remains during Trial 1. Hence, the known ADD of sun exposed

carcasses in this trial was 1.11 times greater than the ADD predicted by the Megyesi et al. [1] formula, when temperatures were recorded at the local BoM weather station.

Linear regression analysis was performed to determine how well the predictive model was estimating the ADD of the remains in the present study. Regression analysis yielded an r^2 value of 0.444, suggesting the method was a poor predictor of the true ADD of sun exposed remains in the current study (approximately 44% accuracy) when using temperatures obtained from the local weather station.
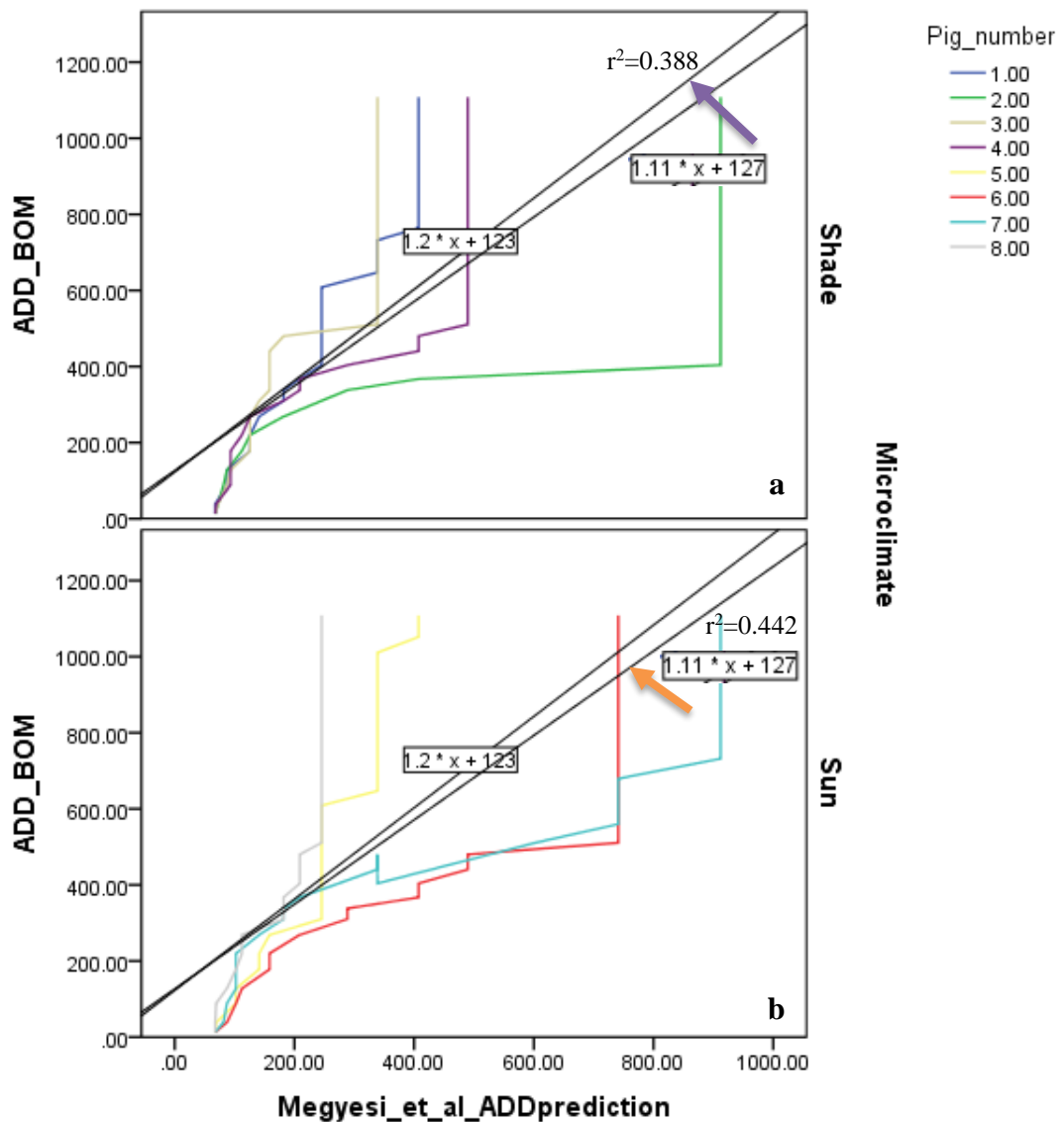
Figure 5-9 The Megyesi et al. [1] prediction of ADD vs. the actual ADD of all carcasses (n=8), where 1.11*x+127 is the line of best fit for the carcasses located in the sun and 1.2*x+123 is the line of best fit for carcasses located in the shade for Trial 1 winter 2014. Units in 'days °C'.

5.5 Validating the Megyesi et al. ADD method- Trial 3

5.5.1 Trial 3 (winter) body scored using TBS

Figure 5-10 depicts the relationship between the TBS assigned, using the Megyesi et al. [1] scoring protocol, for all eight carcasses of Trial 1 of the current study against the known PMI.

A curvilinear pattern is generated when the TBS assigned to the remains in Trial 3 is plotted against the known PMI (Figure 5-10). Decomposition was very slow in this trial and twice stalled for an extended period of time, resulting in the same TBS value being assigned multiple times to the remains. As decomposition changes were not observed, a new TBS value could not be assigned to the carcass. This is observed as a plateau from Day 35 and 50 (as indicated by the blue arrow) and again from Day 55 and 90 (as indicated by the red arrow). This is in contrast to Trial 1 (Figure 5-7), where decomposition progressed steadily over time before the remains dried and mummified, resulting in an exponential pattern when the TBS and PMI are graphed.
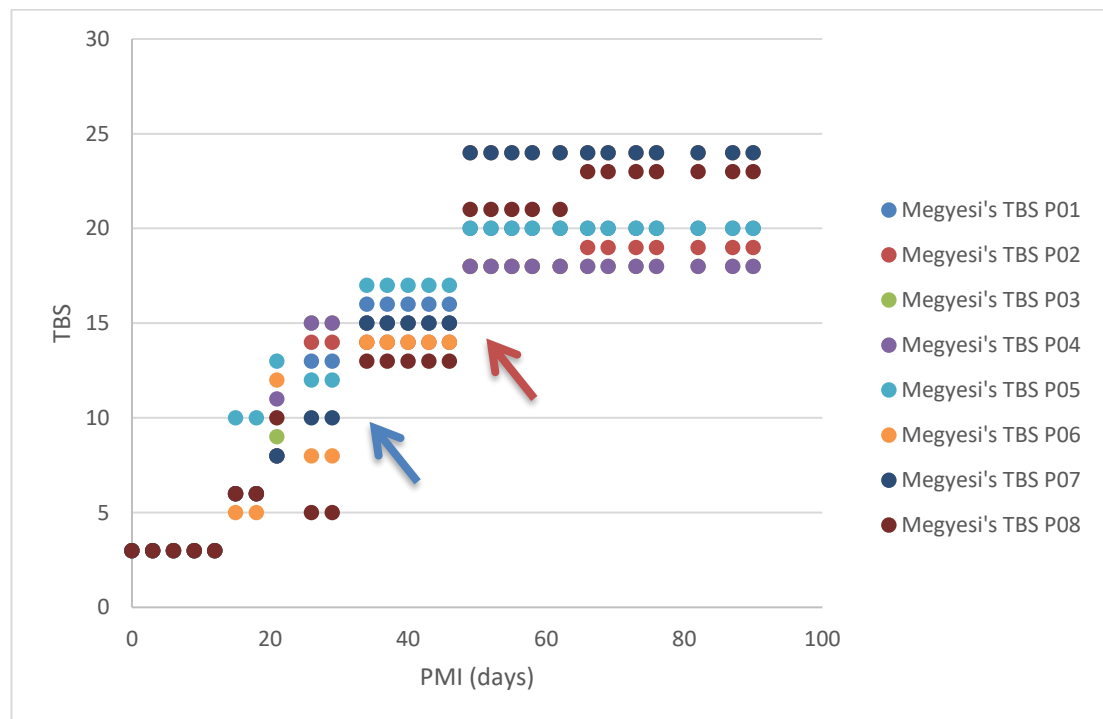
Figure 5-10 Scatterplot of TBS vs. PMI (in days) for all carcasses (Pigs 1-8) to demonstrate the relationship between transpired time and the decomposition of remains for Trial 3. Blue arrow indicates the beginning of a plateau where the TBS did not change between Days 35 and 50. The red arrow indicates a second plateau beginning at Day 55 where the TBS did not change for the remainder of the study.

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5.5.2 Using temperature data collected at the research site

Figure 5-11 depicts the predicted ADD against the known ADD, as recorded by the data loggers at the research site, of remains located in the sun exposed (Figure 5-11a) and shaded (Figure 5-11b) microclimates. As observed in Trial 1, there is a close observable relationship between the known and predicted ADD up until approximately 100ADD. After this point, no clear relationship is observable between the two variables.

Due to software limitations, the line of best fit for the shaded remains and the sun exposed remains appears on both Figure 5-11a and Figure 5-11b. Therefore, the line of best fit for shaded remains is indicated by the purple arrow and the line of best fit for sun exposed remains is indicated by the orange arrow.

5.5.2.1 Sun exposed microclimate

The predicted ADD values found using the Megyesi et al. [1] formula for carcasses in the sun exposed microclimate were compared to the known ADD recorded by the data logger in the sun at the research site, using linear mixed modelling.

The results of the linear mixed modelling showed there was a statistically significant difference between the predicted ADD and the actual ADD recorded during Trial 3 when using the Megyesi et al. [1] predictive method on sun exposed remains with the fixed effect being 0.962 (t (209.7) $=$17.9, $p<$ 0.000). This suggests that, on average, the formula overestimated the true ADD of the remains during Trial 3. Hence, the predicted ADD from the Megyesi et al. [1] formula was 0.962 times greater than the known ADD of sun exposed carcasses, when temperatures were measured at the research site.

Linear regression analysis was performed to determine how well the predicted model was estimating the ADD of the remains in the present study. Regression analysis yielded an r^2 value of 0.811. Using the published guideline that an r^2 value of 0.80 or greater is a reasonably good indicator of accuracy, this would suggest that the Megyesi et al. [1] method has the potential to reasonably determine the ADD in the Hawkesbury region when remains have decomposed in a full sun environment and the temperature at the 'discovery' site is known.

5.5.2.2 Shaded microclimate

The predicted ADD values found using the Megyesi et al. [1] formula for carcasses in the shaded microclimate were compared to the known ADD recorded by the data logger in the shade at the research site, using linear mixed modelling.

The results of the linear mixed modelling showed there was a statistically significant difference between the predicted ADD and the actual ADD recorded during Trial 3 when using Megyesi et al.'s [1] predictive method on the shaded remains. The fixed effect for shaded remains, expressed as the slope, increased from the intercept (0.962; as recorded for sun exposed remains) by 2.11, resulting in a fixed effect of 3.07 (t (208.5) =12.7, p<0.000). The results of the linear mixed model suggest that, on average, the formula underestimated the true ADD of the shaded remains during Trial 3 and the known ADD of shaded carcasses in this trial was 3.07 times greater than the ADD predicted by the Megyesi et al. [1] formula, when temperatures were measured at the research site.

Linear regression analysis was performed to determine how well the predicted model was estimating the ADD of the remains in the present study. Regression analysis yielded an r^2 value of 0.659, suggesting the method, while performing better in this trial in comparison to both Trial 1 microclimates, was a poor predictor of the true ADD of shaded remains in the current study when using temperatures obtained from the 'discovery' site.
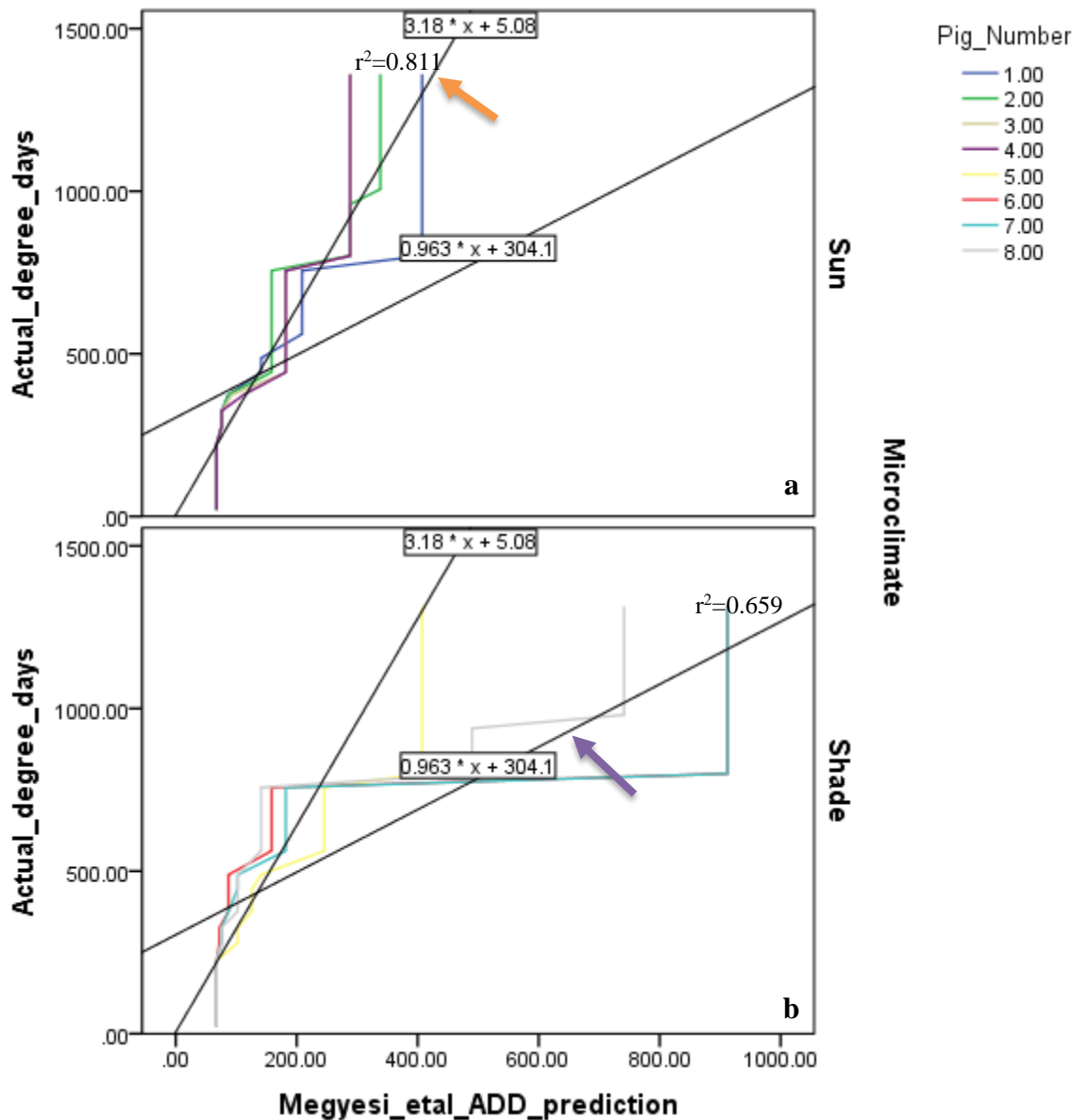
Figure 5-11 The Megyesi et al. [1] prediction of ADD vs. the actual ADD of all carcasses (n=8), where 3.18*x+5.08 is the line of best fit for the carcasses located in the sun and 0.963*x+304.1 is the line of best fit for carcasses located in the shade for Trial 3 winter 2015. Units in 'days °C'.

5.5.3 Using temperature data collected from the BoM weather station

Figure 5-12 depicts the predicted ADD against the known ADD of remains, as recorded by the onsite data loggers, located in the sun exposed (

Figure 5-12a) and shaded (

Figure 5-12b) microclimates. Once again, a clear separation between the known and predicted ADD is observed after approximately 100ADD.

Due to software limitations, the line of best fit for both the shaded remains and the sun exposed remains appears on both
Figure 5-12a and
Figure 5-12b. Therefore, the line of best fit for shaded remains is indicated by the purple arrow and the line of best fit for sun exposed remains is indicated by the orange arrow.

5.5.3.1 Sun exposed microclimate

The predicted ADD values found using the Megyesi et al. [1] formula for carcasses in the sun exposed microclimate were compared to the known ADD recorded by the local BoM weather station, using linear mixed modelling.

The results of the linear mixed modelling showed there was a statistically significant difference between the predicted ADD and the known ADD recorded during Trial 3 when using the Megyesi et al. [1] predictive method on the sun exposed remains, with the fixed effect being 0.782 (t (209.8) =17.6, p<0.000). The results of the linear mixed model suggest that, on average, the formula overestimated the known ADD of the shaded remains during Trial 3 and the ADD predicted by the Megyesi et al. [1] formula was 0.782 times greater than the known ADD of sun exposed remains in the present study, when temperatures were recorded at the local BoM weather station.

Linear regression analysis was performed to determine how well the predicted model was estimating the ADD of the remains in the present study. Regression analysis yielded an r^2 value of 0.790, suggesting that while performing better in this trial in comparison to both Trial 1 microclimates, the method could be a reasonable predictor of the true ADD of sun exposed remains in the current study when using temperatures obtained from the local weather station, with further refinement to the formula.

5.5.3.2 Shaded microclimate

The predicted ADD values found using the Megyesi et al. [1] formula for carcasses in the shaded microclimate were compared to the known ADD recorded by the BoM weather station, using linear mixed modelling.

The results of the linear mixed modelling showed there was a statistically significant difference between the predicted ADD and the actual ADD recorded during Trial 3 when using the Megyesi et al. [1] predictive method on the shaded remains. The

fixed effect for shaded remains, expressed as the slope, increased from the intercept (0.782; as recorded for sun exposed remains) by 0.894, resulting in a fixed effect of 1.67 (t (208.5) =12.3, $p<0.000$). The results of the linear mixed model suggest that, on average, the formula underestimated the true ADD of the shaded remains during Trial 3. Hence, the known ADD of shaded carcasses in this trial was 1.67 times greater than the ADD predicted by the Megyesi et al. [1] formula, when temperatures were measured at the research site.

Linear regression analysis was performed to determine how well the predicted model was estimating the ADD of the remains in the present study. Regression analysis yielded an r^2 value of 0.659, suggesting the method was a poor predictor of the true ADD of shaded remains in the current study when using temperatures obtained from the 'discovery' site.

The results obtained from the linear mixed model and the regression analysis suggest the method performed better during Trial 3 when applying temperatures obtained from the local weather station than when temperatures from the research site are used.
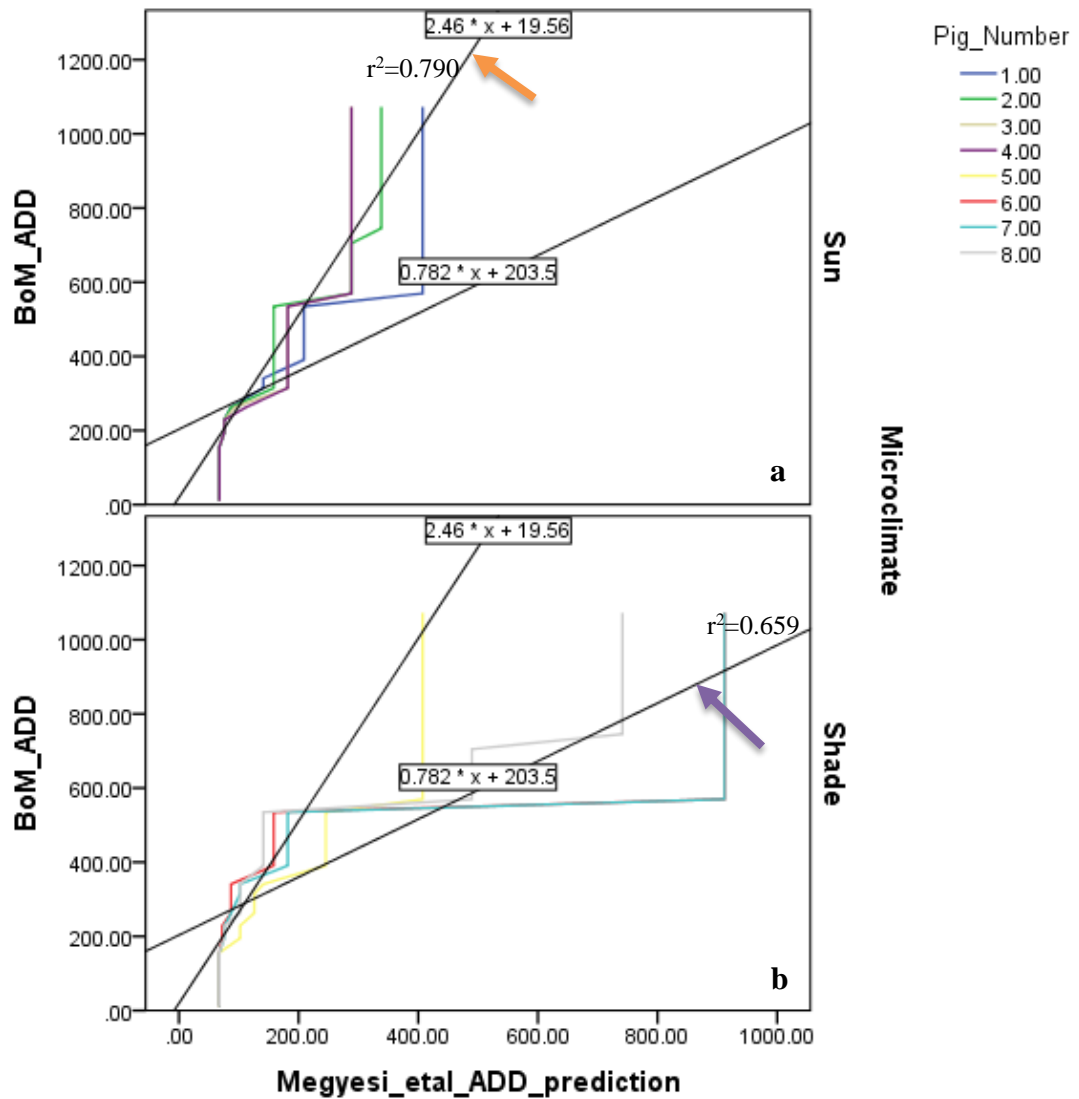
Figure 5-12 The Megyesi et al. [1] prediction of ADD vs. the actual ADD of all carcasses (n=8), where 12.46*x+196.56 is the line of best fit for the carcasses located in the sun and 0.782*x+203.5 is the line of best fit for carcasses located in the shade for Trial 3 winter 2015. Units in 'days °C'.

5.5.4 Summary

The results of the validation of the Megyesi et al. [1] method of determining PMI indicate that largely, this method is not appropriate for accurately determining PMI using the ADD method in a Western Sydney winter climate. This may be due to the known and predicted ADD losing their close relationship after approximately 100ADD. Table 5-1 and Table 5-2 numerically summarises the application of the Megyesi et al. [1] method at randomly selected 'discovery' dates from Trial 1 and Trial 3, respectively. Results are reported from a single carcass (Pig 1; Trial 1 and

Trial 3), with full results reported in Appendix F and Appendix L. The summary tables confirm the pattern seen in Figure 5-8, Figure 5-9, Figure 5-11 and Figure 5-12, where accuracy using the ADD appears to be maintained until approximately 100ADD, after which, the difference between the known and predicted ADD becomes significant and the method is deemed to be inaccurate.

As Table 5-1 and Table 5-2 demonstrates, the difference between the known and predicted ADD at a time point below 100ADD is a matter of hours, while the next time point greater than 100ADD differs by 3-4 days, with the gap increasing as the PMI increases. This is reflected in both winter trials.

Table 5-1 Summary table comparing the predicted ADD with the known ADD for Pig 1 (shade) in Trial 1[*].

PMI (in days)	Predicted ADD[*]:	Known ADD: Data Logger	Predicted ADD[*]: BoM	Known ADD: BoM
5	67.61 (5.78 days)	77.95	67.61 (5.32 days)	78.00
10	87.10 (7.45 days)	140.35	87.10 (6.85 days)	142.15
15	125.89 (10.76 days)	200.90	125.89 (9.91 days)	205.55
40	254.47 (21.76 days)	457.90	254.47 (20.04 days)	469.65
90	407.38 (38.84 days)	1055.35	407.38 (32.07 days)	1108.10

[*]ADD expressed as days °C. The ADD has been converted to days and appears in the brackets within the table.

Table 5-2 Summary table comparing the predicted ADD with the known ADD for Pig 1 (sun) in Trial 3[*].

Day in PMI	Predicted ADD[*]	Known ADD: Data Logger	Predicted ADD[*]: BoM	Known ADD: BoM
5	67.61 (4.52 days)	107.90	67.61 (5.73 days)	74.45
15	75.86 (5.09 days)	277.20	75.86 (6.44 days)	194.95
20	87.10 (5.84 days)	374.05	87.10 (7.39 days)	262.75
40	208.93 (14.02 days)	670.40	208.93 (17.73 days)	468.50
90	407.38 (27.34 days)	1359.80	407.38 (24.58 days)	1072.5

[*] ADD expressed as days °C. The ADD has been converted to days and appears in the brackets within the table.

On average, the published method underestimated the ADD in the two winter trials by approximately 1.5 times the true ADD value, using both temperatures obtained at the research site and the local weather station.

Applying this method using BoM temperatures in Trial 3 appears to better the estimation of ADD than when the temperatures at the research site are used, however, accuracy is still below an accepted level of 80%.

The results of the validation of the Megyesi et al. [1] method will be compared to the other published methods at the conclusion of this chapter.

5.6 Validating the Marhoff et al. predictive equation -Trial 1

5.6.1 Using temperature recorded at the research site

Figure 5-13 depicts the predicted ADD against the known ADD of remains, as recorded by the onsite data loggers, located in the shaded (Figure 5-13a) and sun exposed (Figure 5-13b) microclimates. Both microclimates exhibit similarities in predicted and known ADD until approximately 400ADD, after which, separation between carcasses and the two variables is observed.

Due to software limitations, the line of best fit for both the shaded remains and the sun exposed remains appears on both Figure 5-13a and Figure 5-13b. Therefore, the line of best fit for shaded remains is indicated by the purple arrow and the line of best fit for sun exposed remains is indicated by the orange arrow.

5.6.1.1 Shaded microclimate

The predicted ADD values found using the Marhoff et al. [2] formula for carcasses in the shaded microclimate were compared to the known ADD recorded by the data logger in the shade at the research site.

The results of the linear mixed modelling showed there was a statistically significant difference between the predicted ADD and the actual ADD recorded during Trial 1 when using the Marhoff et al. [2] predictive method on shaded remains. The fixed effect for shaded remains, expressed as the slope, decreased from the intercept (1.71; as recorded for sun exposed remains) by 0.566, resulting in a fixed effect of 1.15 (t (209.7) =-4.34, p<0.000). These results of the linear mixed model suggest that, on average, the formula underestimated the true ADD during Trial 1. Hence, the known ADD of the shaded remains found in this region was 1.15 times greater than the ADD predicted by the Marhoff et al. [2] formula.

Linear regression analysis was performed to determine how well the predicted model was estimating the ADD of the remains in the present study. Regression analysis yielded an r^2 value of 0.660 (approximately 66% accurate), suggesting the method was a poor predictor of the true ADD of shaded remains in the current study when using temperatures obtained from the 'discovery' site.

5.6.1.2 Sun exposed microclimate

The predicted ADD values found using the Marhoff et al. [2] formula for carcasses in the sun exposed microclimate were compared to the known ADD recorded by the data logger in the sun at the research site.

The results of the linear mixed modelling showed there was a statistically significant difference between the predicted ADD and the known ADD recorded during Trial 1 when using the Marhoff et al. [2] predictive method on shaded remains, with the fixed effect being 1.71 (t (210.4) =18.1, $p<0.000$). These results suggest that, on average, the formula underestimated the true ADD during Trial 1. Hence, the known ADD of the shaded remains found in this region was 1.71 times greater than the ADD predicted by the Marhoff et al. [1] formula.

Linear regression analysis was performed to determine how well the predicted model was estimating the ADD of the remains in the present study. Regression analysis yielded an r^2 value of 0.612, suggesting the method was a poor predictor of the true ADD of sun exposed remains in the current study when using temperatures obtained from the 'discovery' site.
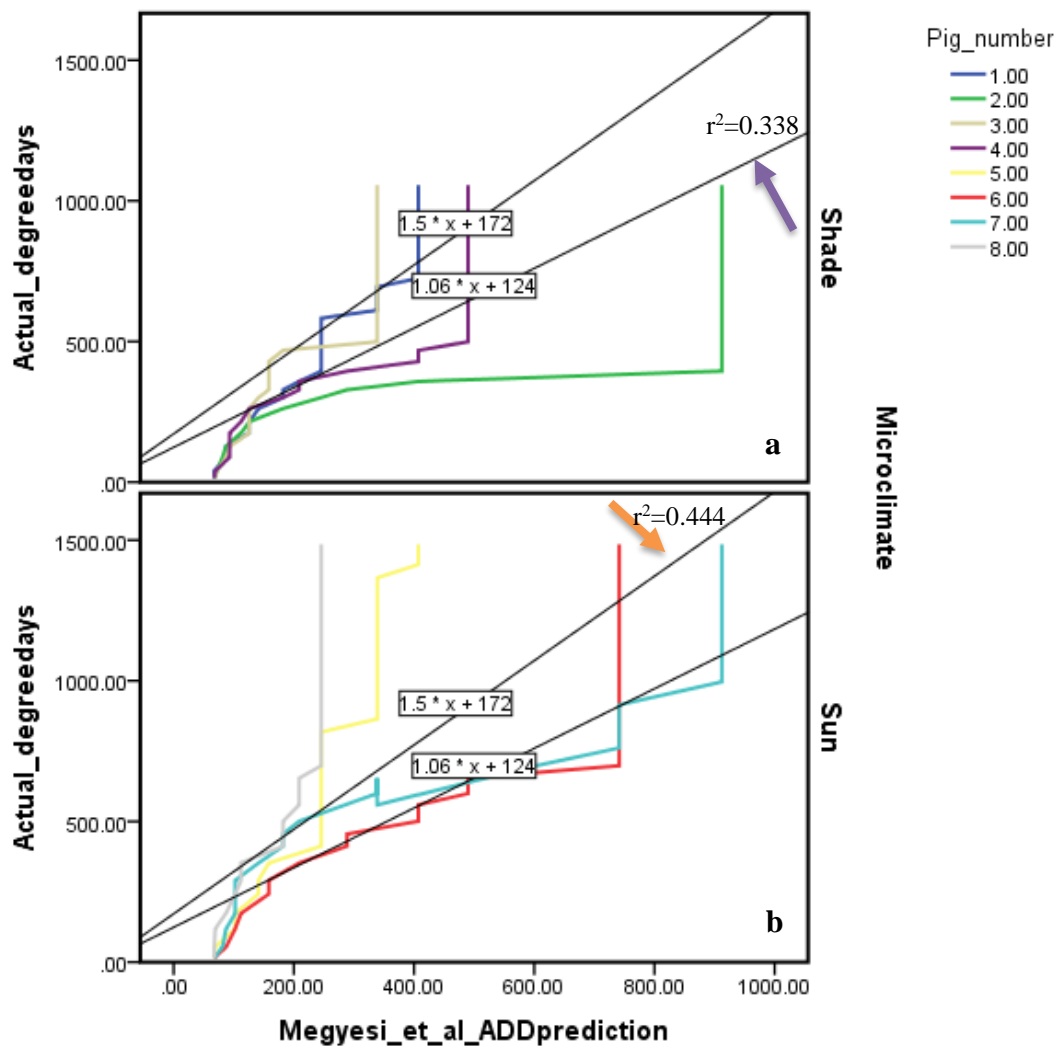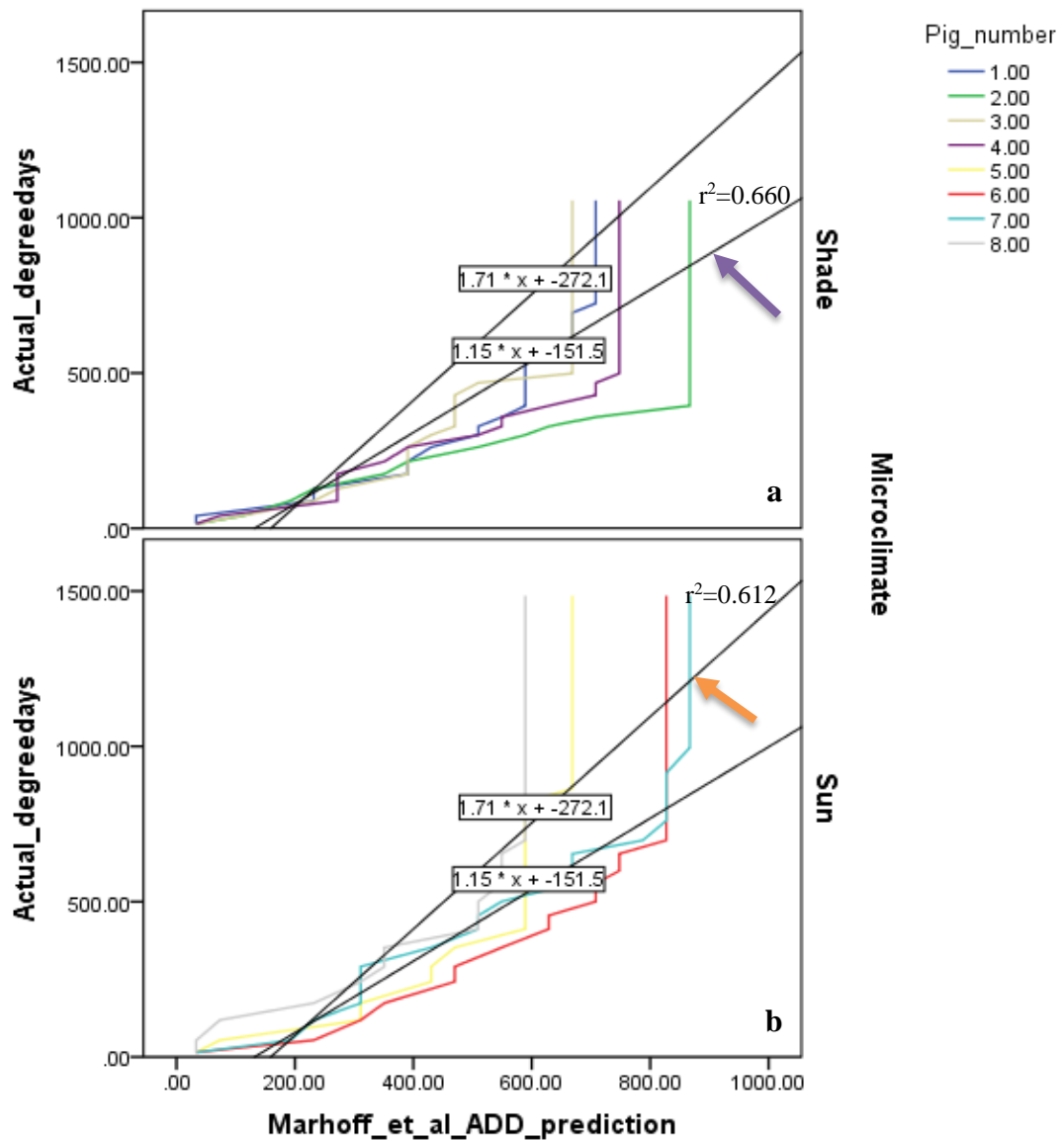
Figure 5-13 The Marhoff et al. [2] ADD prediction vs. the actual ADD of all carcasses (n=8), where 1.15*x+ -151.5 is the line of best fit for carcasses located in the shade and 1.71*x +-272.1 is the line of best fit for carcasses located in the sun for Trial 1 winter 2014. Units in 'days °C'.

5.6.2 Using temperature collected from the BoM weather station

Figure 5-14 depicts the predicted ADD against the known ADD, as recorded by the BoM weather station, for remains located in the shaded (Figure 5-14a) and sun exposed (Figure 5-14b) microclimates. Both microclimates exhibit similarities in predicted and known ADD until approximately 400ADD, after which, separation between carcasses and the two variables is observed.

Due to software limitations, the line of best fit for both the shaded remains and the sun exposed remains appears on both Figure 5-14a and Figure 5-14b. Therefore, the line of best fit for shaded remains is indicated by the purple arrow and the line of best fit for sun exposed remains is indicated by the orange arrow.

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5.6.2.1 Shaded microclimate

The predicted ADD values found using the Marhoff et al. [2] formula for carcasses in the shaded microclimate were compared to the known ADD recorded by the BoM weather station, using linear mixed modelling.

The results of the linear mixed modelling showed there was not a statistically significant difference between the predicted ADD and the actual ADD recorded during Trial 1 when using the Marhoff et al. [2] predictive method on the shaded remains. The fixed effect for shaded remains, expressed as the slope, decreased from the intercept (1.26; as recorded for sun exposed remains) by 0.408, resulting in a fixed effect of 0.802 (t (210.1) =-0.408, $p<0.640$). The results of the linear mixed model suggest that, on average, the formula overestimated the true ADD of the shaded remains during Trial 1. Hence, the predicted ADD of carcasses in this trial was approximately 0.802 times greater than the known ADD value.

Linear regression analysis was performed to determine how well the predicted model was estimating the ADD of the remains in the present study. Regression analysis yielded an r^2 value of 0.656, suggesting the method was a poor predictor of the true ADD of shaded remains in the current study when using temperatures obtained from the BoM weather station.

5.6.2.2 Sun exposed microclimate

The predicted ADD values found using the Marhoff et al. [2] formula for carcasses in the sun exposed microclimate were compared to the known ADD recorded by the local BoM weather station, using linear mixed modelling.

The results of the linear mixed modelling showed there was a statistically significant difference between the predicted ADD and the known ADD recorded during Trial 1 when using the Marhoff et al. [2] predictive method on the sun exposed remains, with the fixed effect being 1.26 (t (210.7) =15.5, $p<0.001$). The results of the linear mixed model suggest that, on average, the formula underestimated the true ADD of the sun exposed remains during Trial 1. Hence, the known ADD of carcasses in this trial, as recorded by the BoM weather station, was 1.26 times greater than the ADD predicted by the Marhoff et al. [2] formula.

Linear regression analysis was performed to determine how well the predicted model was estimating the ADD of the remains in the present study. Regression analysis yielded an r^2 value of 0.608, suggesting the method was a poor predictor of the true ADD of sun exposed remains in the current study when using temperatures obtained from the local weather station.
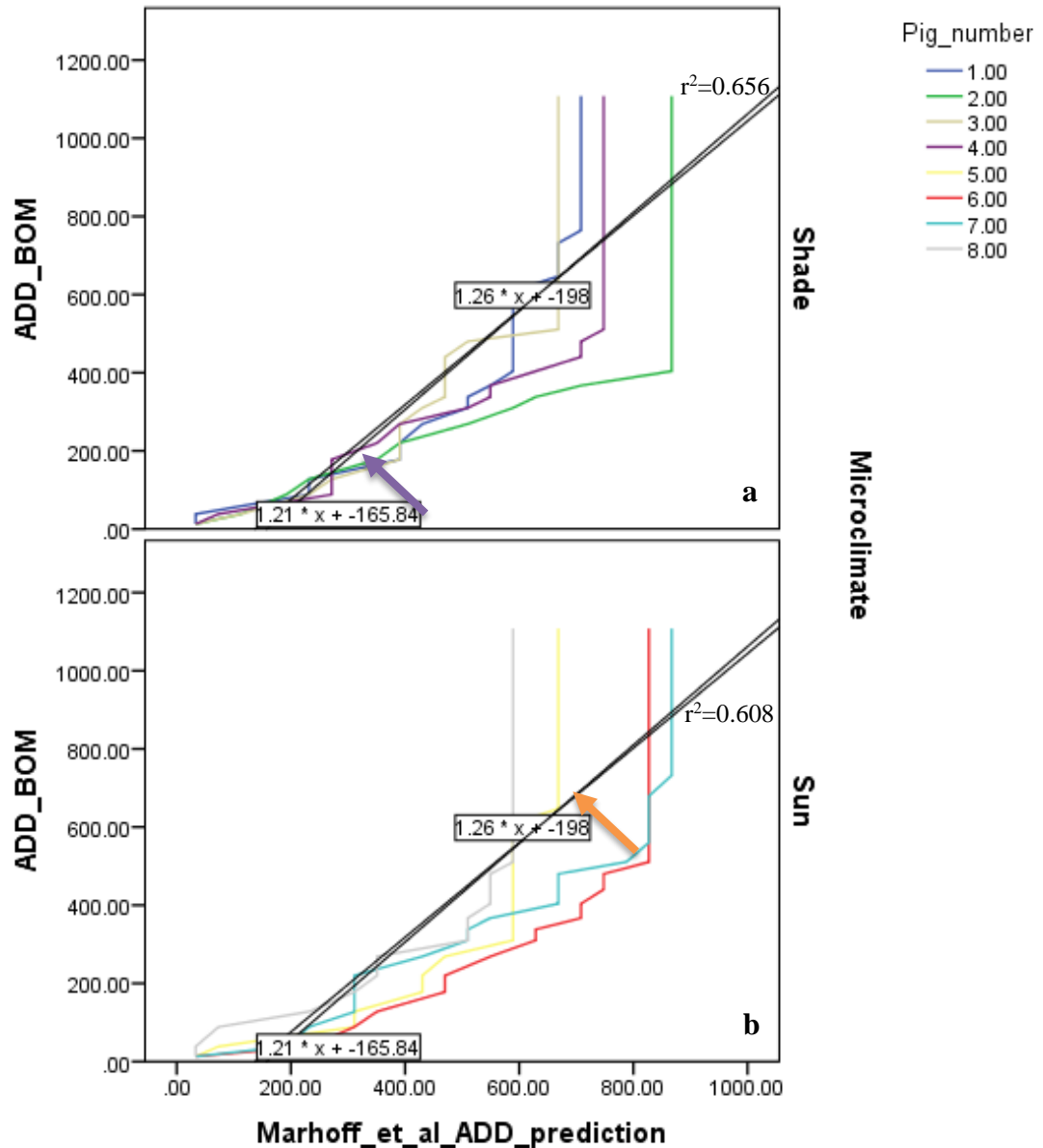
**Figure 5-14 The Marhoff et al. [2] ADD prediction vs. the known ADD of all carcasses (n=8) found using local weather station temperature for carcasses located in the sun and shade for Trial 1 winter. Where the line of best fit for PMI shade=1.21*x+-165.8 and PMI sun= 1.26*x+-198. Units in 'days °C'.**

5.7   Validating the Marhoff et al. predictive equation -Trial 3

5.7.1   Using temperature recorded at the research site

Figure 5-15 depicts the predicted ADD against the known ADD of remains, as recorded by the onsite data loggers, located in the sun exposed (Figure 5-15a) and shaded (Figure 5-15b) microclimates. Both microclimates exhibit similarities in predicted and known ADD until approximately 700ADD, after which, separation between carcasses and the two variables is observed.

Due to software limitations, the line of best fit for both the shaded remains and the sun exposed remains appears on both Figure 5-15a and Figure 5-15b. Therefore, the line of best fit for shaded remains is indicated by the purple arrow and the line of best fit for sun exposed remains is indicated by the orange arrow.

5.7.1.1   Shaded microclimate

The predicted ADD values found using the Marhoff et al. [2] formula for carcasses in the shaded microclimate were compared to the known ADD recorded by the data logger in the shade at the research site.

The linear mixed modelling demonstrated a statistically significant difference between the predicted ADD and the actual ADD recorded during Trial 3 when using the Marhoff et al. [2] formula on shaded remains. The fixed effect for shaded remains, expressed as the slope, increased from the intercept (1.11; as recorded for sun exposed remains) by 0.291, resulting in a fixed effect of 1.40 (t (212) =3.99, p<0.000). These results suggest that, on average, the formula underestimated the true ADD during Trial 3 and the known ADD of the shaded remains found in this region was 1.40 times greater than the ADD predicted by the Marhoff et al. [2] formula.

Linear regression analysis was performed to determine how well the predicted model was estimating the ADD of the remains in the present study. Regression analysis yielded an r^2 value of 0.863. Using the published guideline that an r^2 value of 0.80 or greater is a reasonably good indicator of accuracy, this would suggest that the Marhoff et al. [2] formula has the potential to reasonably determine the ADD in the Hawkesbury region when remains have decomposed in a shaded environment and the temperature at the 'discovery' site is known.

5.7.1.2 Sun exposed microclimate

The predicted ADD values found using the Marhoff et al. [2] formula for carcasses in the sun exposed microclimate were compared to the known ADD recorded by the data logger in the sun at the research site.

The linear mixed modelling demonstrated a statistically significant difference between the predicted ADD and the known ADD recorded during Trial 3 when using the Marhoff et al. [2] predictive method on sun exposed remains, with the fixed effect being 1.11 (t (212) =24.8, p<0.000). These results suggest that, on average, the formula underestimated the true ADD during Trial 1 and the known ADD of the sun exposed remains found in this region was 1.11 times greater than the ADD predicted by the Marhoff et al. [2] formula.

Linear regression analysis was performed to determine how well the predicted model was estimating the ADD of the remains in the present study. Regression analysis yielded an r^2 value of 0.832. Using the published guideline that an r^2 value of 0.80 or greater is a reasonably good indicator of accuracy, this would suggest that the Marhoff et al. [2] method has the potential to reasonably determine the ADD in the Hawkesbury region when remains have decomposed in a sun exposed environment and the temperature at the 'discovery' site is known.
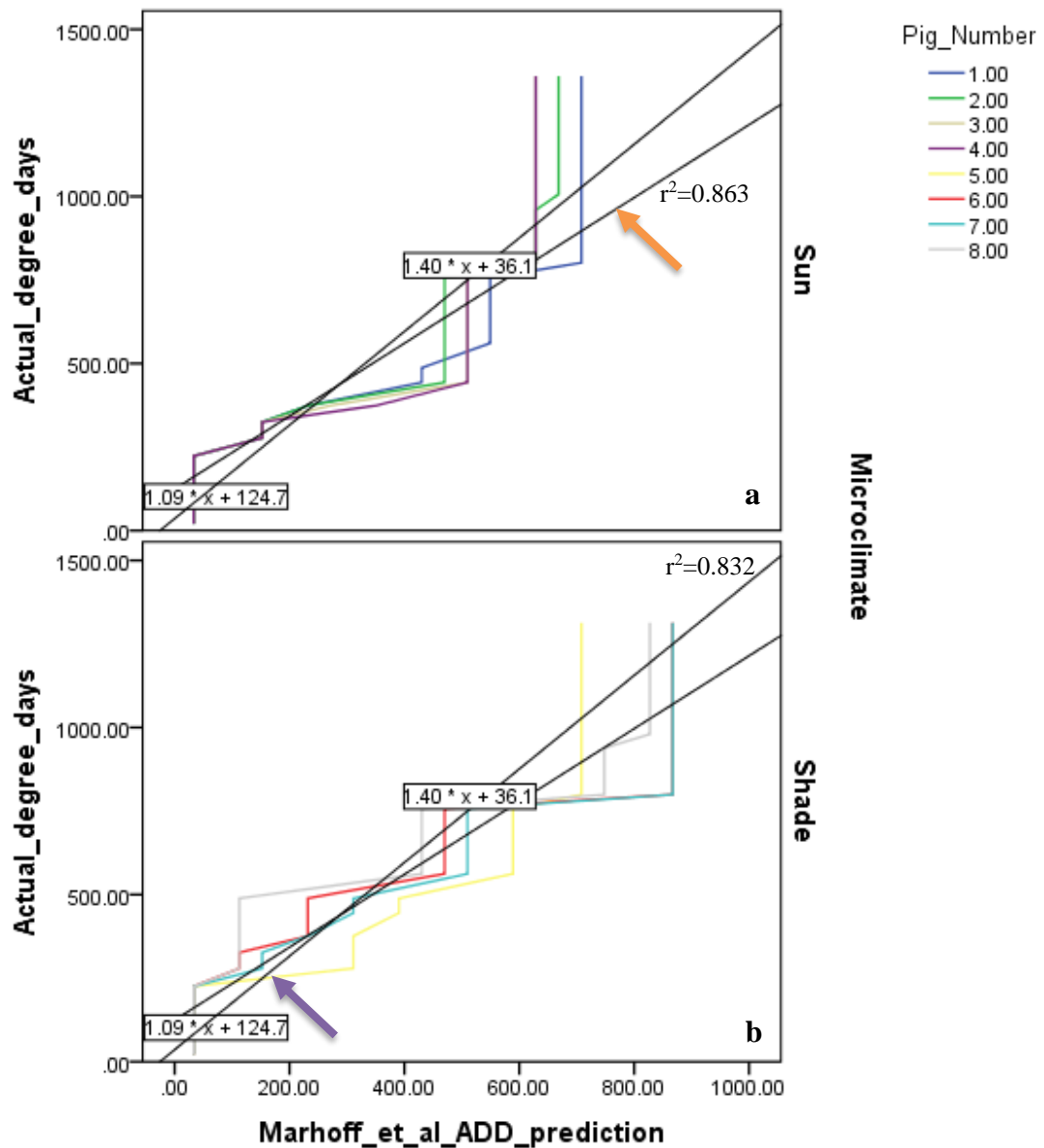
Figure 5-15 The Marhoff et al. [2] ADD prediction vs. the actual ADD of all carcasses (n=8) found using data logger temperature for carcasses located in the sun and for carcasses located in the shade for winter trial 3 2015. Where the line of best fit for PMI shade=1.09*x+124.7; PMI sun= 1.40*x+36.1. Units in 'days °C'.

5.7.2 Using temperature data collected from the BoM weather station

Figure 5-16 depicts the predicted ADD against the known ADD, as recorded by the BoM weather station, for remains located in the sun exposed (Figure 5-16a) and shaded (Figure 5-16b) microclimates. Both microclimates exhibit similarities in predicted and known ADD until approximately 700ADD, after which, separation between carcasses and the two variables is observed.

Due to software limitations, the line of best fit for both the shaded remains and the sun exposed remains appears on both Figure 5-16a and Figure 5-16b. Therefore, the

line of best fit for shaded remains is indicated by the purple arrow and the line of best fit for sun exposed remains is indicated by the orange arrow.

5.7.2.1 Sun exposed microclimate

The predicted ADD values found using the Marhoff et al. [2] formula for carcasses in the sun exposed microclimate were compared to the known ADD recorded by the local BoM weather station.

The linear mixed modelling demonstrated a statistically significant difference between the predicted ADD and the known ADD recorded during Trial 3 when using the Marhoff et al. [2] formula on sun exposed remains, with the fixed effect being 0.782 (t (209.8) =17.6, p<0.000). These results suggest that, on average, the formula [2] overestimated the true ADD during Trial 3 and the Marhoff et al. [2] formula was estimating the known ADD to be 0.782 times greater than the true ADD value.

Linear regression analysis was performed to determine how well the predicted model was estimating the ADD of the remains in the present study. Regression analysis yielded an r^2 value of 0.777, suggesting the method could be a good predictor of the true ADD of sun exposed remains in the current study with further calibration to account for all variables affecting decomposition.

5.7.2.2 Shaded microclimate

The predicted ADD values found using the Marhoff et al. [2] formula for carcasses in the shaded microclimate were compared to the known ADD recorded by the local BoM weather station.

The linear mixed modelling demonstrated a statistically significant difference between the predicted ADD and the known ADD recorded by the BoM weather station during Trial 3 when using the Marhoff et al. [2] formula on shaded remains. The fixed effect for shaded remains, expressed as the slope, increased from the intercept (0.782; as recorded for sun exposed remains) by 1.67, resulting in a fixed effect of 2.45 (t (208.5) =12.3, p<0.000). These results suggest that, on average, the formula underestimated the true ADD during Trial 3. Hence, the known ADD of the shaded remains found in this region was 2.45 times greater than the ADD predicted by the Marhoff et al. [2] formula.

Linear regression analysis was performed to determine how well the predicted model was estimating the ADD of the remains in the present study. Regression analysis yielded an r^2 value of 0.835. Using the published guideline that an r^2 value of 0.80 or greater is a reasonably good indicator of accuracy, this would suggest that the Marhoff et al. [2] method has the potential to reasonably determine the ADD in the Hawkesbury region when remains have decomposed in a shaded environment and the temperature is obtained by the closest weather station.
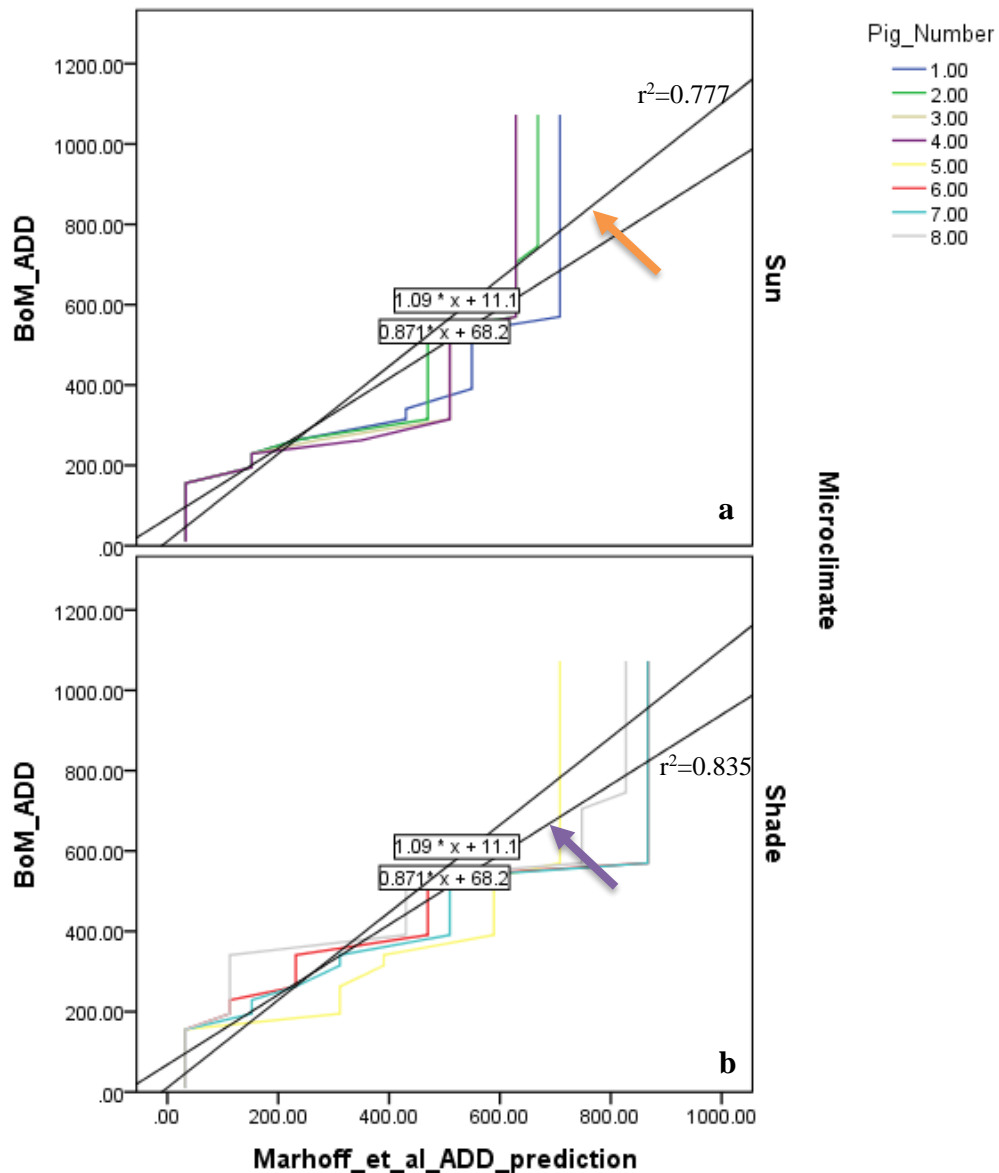
Figure 5-16 The Marhoff et al. [2] ADD prediction vs. the actual ADD of all carcasses (n=8) found using data logger temperature for carcasses in the shade and sun exposed microclimates of Trial 3 (winter). Where the line of best fit for PMI sun= 1.09*x+11.1; PMI shade=0.871*x+68.2. Units in 'days °C'.

5.7.3 Summary

The results of the validation of the Marhoff et al. [2] formula of determining PMI indicate that largely, this method is not appropriate for accurately determining PMI using the ADD method in a Western Sydney winter climate. Table 5-3 and Table 5-4 numerically summarises the application of the Marhoff et al. [2] formula at randomly selected 'discovery' dates from Trial 1 and Trial 3, respectively. Results are reported

from a single carcass (Pig 1; Trial 1 and Trial 3), with full results reported in Appendix G and Appendix M.

Summary Table 5-3 confirms the pattern seen in Figure 5-13 and Figure 5-14, where accuracy using the ADD appears to be maintained until approximately 100ADD, after which, the difference between the known and predicted ADD increases and the method is deemed to be inaccurate.

Summary Table 5-4 confirms the pattern observed in Figure 5-15 and Figure 5-16, where accuracy using the ADD method is maintained between 250-700ADD. After this period, the formula is compromised as decomposition stalls and the formula failed to accurately estimate the correct ADD.

Table 5-3 and Table 5-4 demonstrates, the difference between the known and predicted ADD. The method had the potential to be a reasonable predictor of the PMI when it was applied in Trial 3, particularly when using temperatures obtained from the local BoM weather station. The method performed better in Trial 1 when temperatures from the research site were used for the PMI calculation; however, Trial 3 performed better overall.

Table 5-3 Summary table comparing the predicted ADD with the known ADD for Pig 1 (shade) in Trial 1[*].

PMI (in days)	Predicted ADD[*]:	Known ADD: Data Logger	Predicted ADD[*]: BoM	Known ADD: BoM
5	33.30 (2.84 days)	77.95	33.30 (2.62 days)	78.00
10	231.80 (19.82 days)	140.35	231.80 (18.25 days)	142.15
15	390.60 (33.41 days)	200.90	390.60 (30.75 days)	205.55
40	589.10 (50.39 days)	457.90	589.10 (46.38 days)	469.65
60	668.50 (57.18 days)	673.50	668.50 (52.63 days)	711.00
90	708.20 (60.58 days)	1055.35	708.2 (55.76 days)	1108.10

[*] ADD expressed as days °C. The ADD has been converted to days and appears in the brackets within the table.

Table 5-4 Summary table comparing the predicted ADD with the known ADD for Pig 1 (sun) in Trial 3[*].

Day in PMI	Predicted ADD[*]: Data logger	Known ADD: Data Logger	Predicted ADD[*]: BoM	Known ADD: BoM
5	33.30 (2.31 days)	109.40	33.30 (2.82 days)	74.45
15	152.40 (10.58 days)	279.15	152.40 (12.93 days)	194.95
20	231.80 (16.09 days)	357.75	231.80 (19.67 days)	251.40
40	549.40 (38.15 days)	655.10	549.40 (46.63 days)	456.85
60	708.20 (49.18 days)	915.35	708.2 (60.11 days)	681.70
90	708.20 (49.18 days)	1313.14	708.20 (60.11 days)	1072.50

[*] ADD expressed as days °C. The ADD has been converted to days and appears in the brackets within the table.

On average, the published method [2] underestimated the ADD in the two winter trials by approximately 1.3 times the true ADD value, using both temperatures obtained at the research site and the BoM weather station. Applying this method using onsite data logger temperatures in Trial 3 appears to better the estimation of ADD than when the temperatures from the BoM weather station are used.

The results of the validation of the Marhoff et al. [2] method will be compared to the other published methods at the conclusion of this chapter.

5.8   Validating the Vass Universal PMI Formula-Trial 1

5.8.1   Trial 1 (winter) body scored using percentage of decomposition

Figure 5-17 displays the results of the relationship between the percentage of the overall decay on each of the eight carcasses in Trial 1 of the current study, using the Vass [3] method and time elapsed since death (PMI) as a means of depicting soft tissue changes semi-quantitatively.

The percentage of decay observed on remains increased at a relatively steady rate throughout the length of the trial (90 days), creating an exponential pattern when plotted. At times when decomposition stalled and morphological changes were not observed, a new percentage value could not be assigned to the carcasses resulting in a plateau, as indicated by the blue arrow on Figure 5-17.

Carcasses reached a state of mummification/desiccation by Day 85 (blue arrow) and as a result no further decomposition changes were observed. Observations of each carcass, however, continued to Day 180, despite the primary trial ending at Day 90, to determine the long term stasis of decomposition changes on PMI calculations.
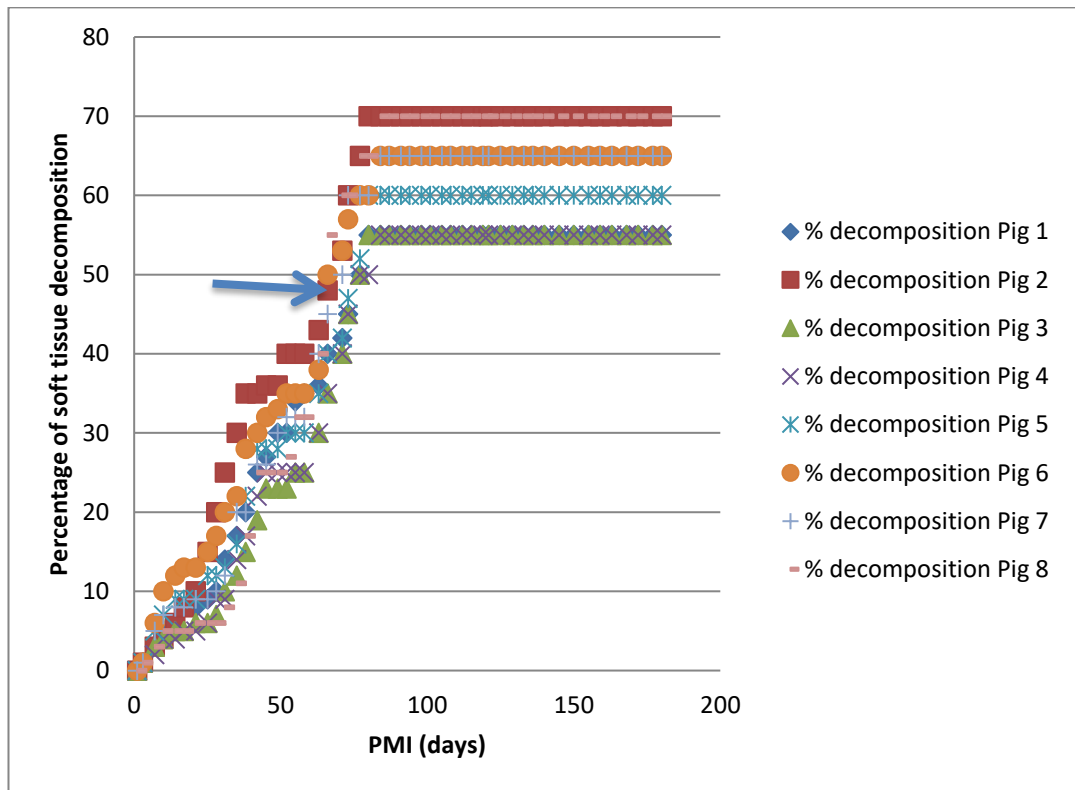
Figure 5-17 Scatterplot of the percentage of soft tissue decomposition vs. PMI to demonstrate decay rates over time for all eight carcasses of Trial 1. The blue arrow indicates the beginning of a plateau from Day 85 when the percentage of decay did not change for the remainder of the study and beyond.

Figure 5-18 depicts the predicted PMI against the known PMI of remains located in the shade (Figure 5-18a) and sun exposed (Figure 5-18b) microclimates, where PMI is measured in days. Both microclimates exhibit similarities in predicted and known PMI until approximately 60 days in the PMI, after which, some separation between individual carcasses and between the two variables is observed.

Due to software limitations, the line of best fit for the shaded remains and the sun exposed remains appears on both Figure 5-18a and Figure 5-18b. Therefore, the line of best fit for shaded remains is indicated by the purple arrow and the line of best fit for sun exposed remains is indicated by the orange arrow.

5.8.2 Shaded microclimate

The estimated PMI found using the Vass [3] formula for carcasses in the shaded microclimate were compared to the known PMI of the remains in Trial 1 of the current study using linear mixed modelling analysis.

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The linear mixed modelling demonstrated a statistically significant difference between the estimated PMI and the known PMI of the shaded remains in Trial 1 found using the Vass [3] predictive formula. The fixed effect for shaded remains, expressed as the slope, decreased from the intercept (0.972; as recorded for sun exposed remains) by 0.214, resulting in a fixed effect of 0.758 (t (205.6) =-6.74, p<0.000). These results suggest that, on average, the formula overestimated the true PMI during Trial 1. Hence, the Vass [3] formula predicted the PMI to be 0.758 times greater than the known PMI value.

Linear regression analysis was performed to determine how well the predicted model was estimating the PMI of the remains in the present study. Regression analysis yielded an r^2 value of 0.884. Using the published guideline that an r^2 value of 0.80 or greater is a reasonably good indicator of accuracy, this would suggest that the Vass [3] formula has the potential to reasonably determine the PMI of carcasses in the Hawkesbury region when remains have decomposed in a shaded environment and the temperature is obtained at the 'discovery' site.

5.8.3 Sun exposed microclimate

The predicted PMI values found using the Vass [3] formula for carcasses in the sun exposed microclimate were compared to the known PMI of the current study, using linear mixed modelling analysis.

The linear mixed modelling demonstrated a statistically significant difference between the predicted PMI and the true PMI of the carcasses in Trial 1 found using the Vass [3] predictive formula on sun exposed remains, with the fixed effect being 0.972 (t (205.2) =38.7, p<0.000). These results suggest that, on average, the formula overestimated the true PMI during Trial 1. Hence, the Vass [3] formula predicted the PMI to be 0.972 times greater than the known PMI value.

Linear regression analysis was performed to determine how well the predicted model was estimating the PMI of the remains in the present study. Regression analysis yielded an r^2 value of 0.928. Using the published guideline that an r^2 value of 0.80 or greater is a good indicator of accuracy, this would suggest that the Vass [3] formula has the potential to reasonably determine the PMI of carcasses in the Hawkesbury region when remains have decomposed in a sun exposed environment and the temperature is obtained at the 'discovery' site.
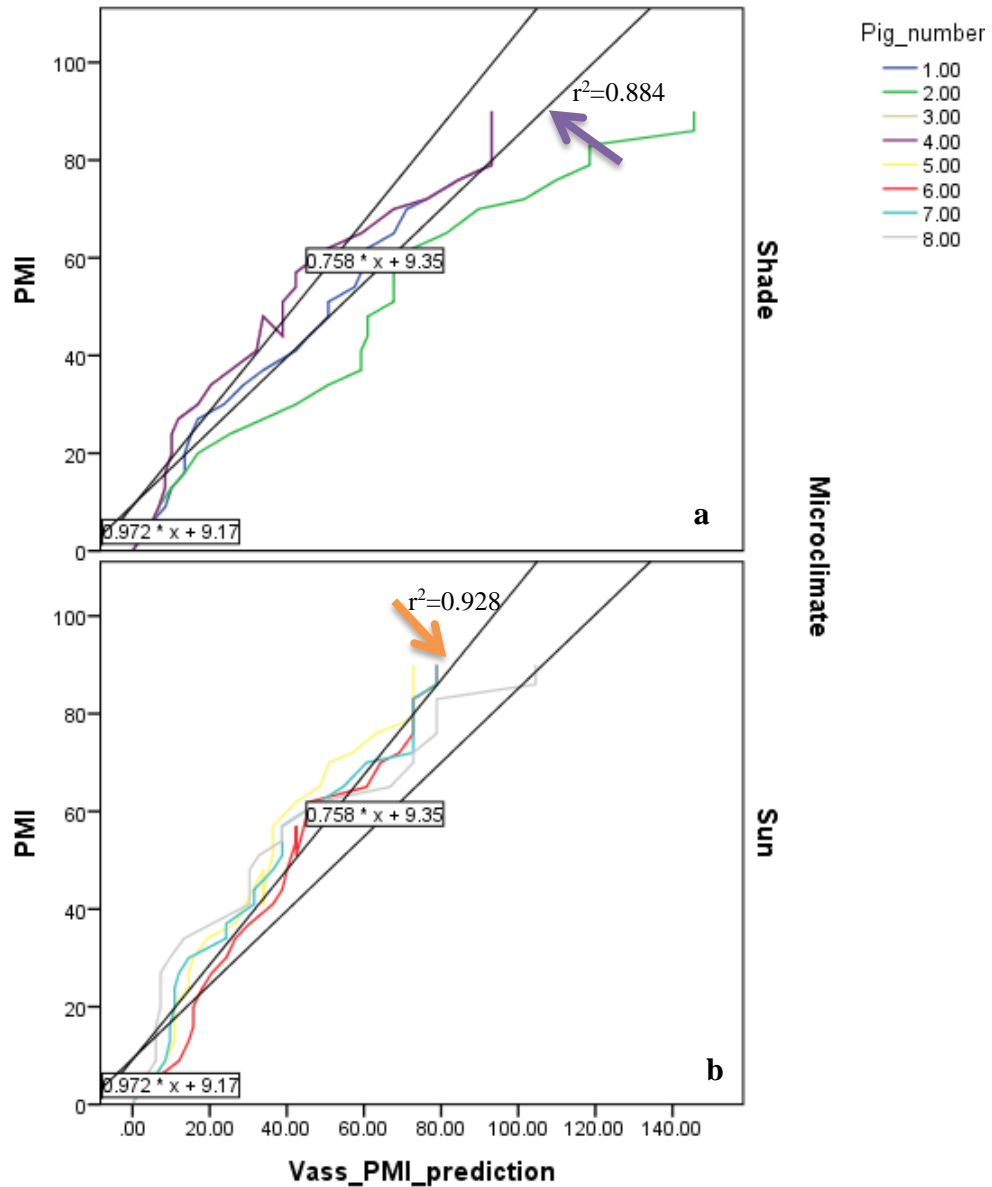
Figure 5-18 The Vass [3] prediction of PMI vs. the actual PMI of all carcasses (n=8) to demonstrate the relationship between the actual and predicted PMI of the carcasses in the sun and the shade. Chart includes the line of best for each microclimate. PMI shade= 0.972*x+9.17; PMI sun= 0.758*x+9.35. Units are in 'days'.

5.9   Validating the Vass Universal PMI Formula-Trial 3

5.9.1   Trial 3 (winter) body scored using percentage of decomposition

Figure 5-19 displays the results of the relationship between the percentage of overall decay on each of the eight carcasses in Trial 3 of the current study, using the Vass [3] formula, and PMI as a means of depicting soft tissue changes semi-quantitatively. Figure 5-19 demonstrates that decomposition of soft tissue occurred more slowly during Trial 3 compared to Trial 1 (Figure 5-17). It can be seen that while the

percentage of decay was increasing overtime, there were extensive periods when the decay process stalled creating a curvilinear pattern. This is depicted as a series of plateaus on the graph (as indicated by the arrows). The first plateau occurred at varied times for each carcass, with decomposition stalling as early as Day 25 (red arrow) and as late as Day 49 (blue arrow).

This is in contrast to Trial 1 (Figure 5-17) which showed a steady increase in soft tissue decay as time transpired. Almost all carcasses reached a third plateau around Day 70 (purple arrow). The overall percentage of decay did not change after this time point for the duration of the study. Again, the degree of decay was observed beyond the 90 day trial period through to Day 180.
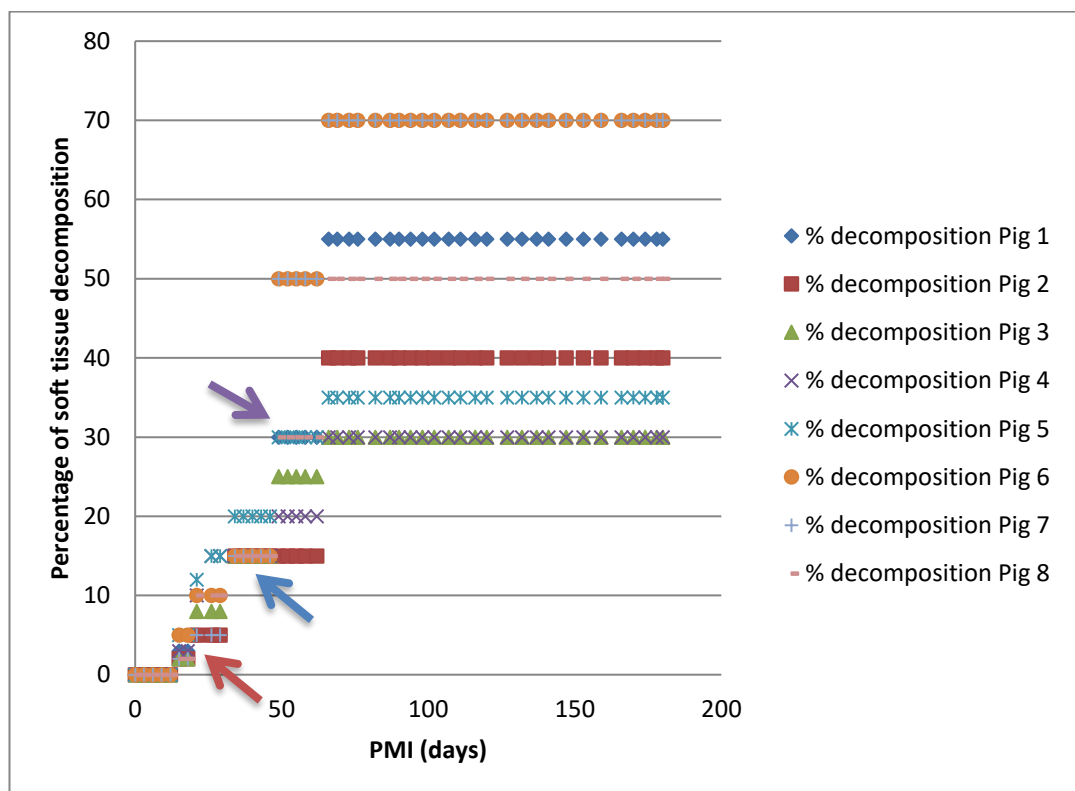
Figure 5-19 Scatterplot of the percentage of soft tissue decomposition vs. PMI to demonstrate decay rates over time for all eight carcasses of Trial 3. Red arrow indicates the beginning of a plateau at Day 25. Blue arrow depicts a second plateau at Day 49. Purple arrow indicates a final plateau at Day 70 where the percentage of decay did not change for the remainder of the trial (Day 90) and beyond (Day 180).

Figure 5-20 depicts the predicted PMI against the known PMI of remains located in the sun exposed (Figure 5-20a) and shaded (Figure 5-20b) microclimates, where PMI is measured in days.

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Due to software limitations, the line of best fit for the shaded remains and the sun exposed remains appears on both Figure 5-20a and Figure 5-20b. Therefore, the line of best fit for shaded remains is indicated by the purple arrow and the line of best fit for sun exposed remains is indicated by the orange arrow.

5.9.2 Sun exposed microclimate

The estimated PMI using the Vass [3] formula for carcasses in the sun exposed microclimate was compared to the known PMI of the remains in Trial 3 of the current study using linear mixed modelling analysis.

The linear mixed modelling demonstrated a statistically significant difference between the estimated PMI and the true PMI of the sun exposed remains in Trial 3 when using the Vass [3] formula, with the fixed effect for sun exposed remains being 0.636 (t (208.2) =22.9, p<0.000). These results suggest that, on average, the formula overestimated the true PMI during Trial 3 and the Vass [3] formula predicted the PMI to be 0.636 times greater than the known PMI value.

Linear regression analysis was performed to determine how well the predicted model was estimating the PMI of the remains in the present study. Regression analysis yielded an r^2 value of 0.807. Using the published guideline that an r^2 value of 0.80 or greater is a reasonably good indicator of accuracy, this would suggest that Vass' [3] method has the potential to reasonably determine the PMI of carcasses in the Hawkesbury region when remains have decomposed in a shaded environment and the temperature is obtained at the 'discovery' site.

5.9.3 Shaded microclimate

The estimated PMI using the Vass [3] formula for carcasses in the shaded microclimate was compared to the known PMI of the remains in Trial 3 of the current study using linear mixed modelling analysis.

The linear mixed modelling demonstrated a statistically significant difference between the estimated PMI and the true PMI of the shaded remains in Trial 3 when using the Vass [3] predictive formula. The fixed effect for shaded remains, expressed as the slope, increased from the intercept (0.972; as recorded for sun exposed remains) by 0.306, resulting in a fixed effect of 0.943 (t (208) =6.19, p<0.000). These results suggest that, on average, the formula overestimated the true PMI

during Trial 3 and the Vass [3] formula predicted the PMI to be 0.943 times greater than the known PMI value.

Linear regression analysis was performed to determine how well the predicted model was estimating the PMI of the remains in the present study. Regression analysis yielded an r^2 value of 0.795, suggesting the method could be a reasonable predictor of the true PMI of shaded remains in the current study.
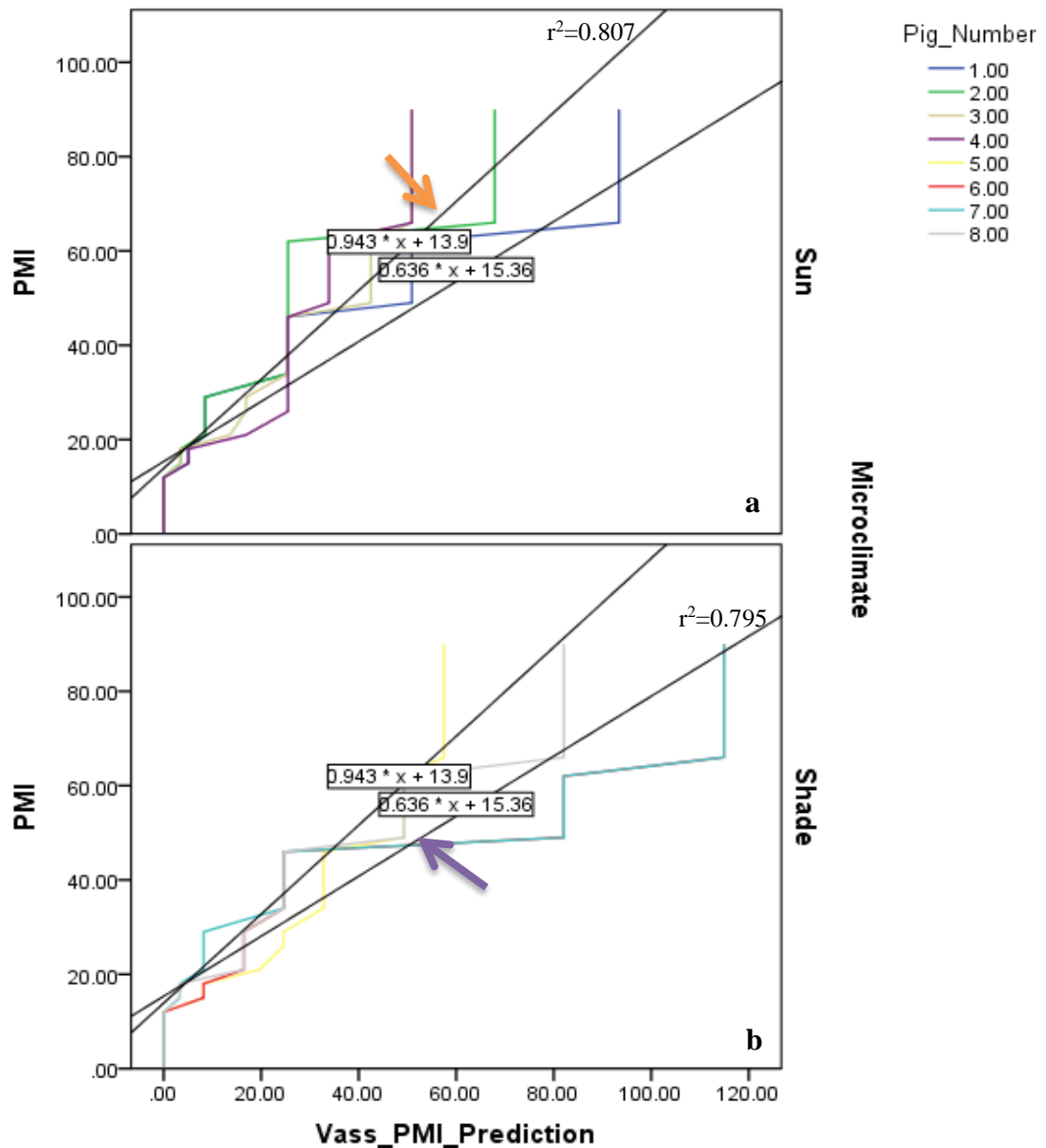
Figure 5-20 The Vass [3] PMI prediction vs. the actual PMI of all carcasses (n=8) to demonstrate the relationship between the actual and predicted PMI of the carcasses in the sun and the shade. Chart includes the line of best for each microclimate. PMI sun= 0.943*x+13.9; PMI shade=0.636*x+15.36. Units are in 'days'.

5.9.4 Summary

The results of the validation of the Vass [3] formula for determining PMI, indicate varied results when the formula was applied across the two winter seasons. Therefore, this method is not appropriate for accurately determining PMI using the Vass [3] formula in a Western Sydney winter climate. This may be due to a change in environmental variables across the two seasons from 2014 to 2015 (e.g. moisture levels). Table 5-5 numerically summarises the application of the Vass [3] formula at randomly selected 'discovery' dates from Trial 1 and Trial 3. Results are reported from a single carcass (Pig 1; Trial 1 and Trial 3), with full results reported in Appendix H and Appendix N.

The summary table confirms the pattern seen in Figure 5-18 and Figure 5-20, where accuracy using the formula appears to be maintained until approximately Day 60 in Trial 1, however, when this method was applied in Trial 3, the predicted and known PMI are rarely comparable at any time point.

Table 5-5 Summary table comparing the predicted PMI with the known PMI for Pig 1 (shade) in Trial 1 and Pig 1 (sun) in Trial 3.

PMI (in days)	Predicted PMI (days): Trial 1	Predicted PMI (days): Trial 3
5	1.69	0
10	8.47	0
15	10.16	5.10
40	33.86	25.50
90	93.12	93.40

On average, the published method [3] overestimated the PMI in the two winter trials by approximately 0.827 times the true PMI value. The Vass [3] formula closely predicted the true PMI of the remains, as found by the adjusted r^2 value, at many time points across Trial 1 was concluded to be the most accurate of the methods tested in the winter trials for the current study.

5.10 Discussion

Initially, the difference between the predicted ADD generated by the Megyesi et al. [1] method and the true ADD recorded by both the onsite data loggers and the BoM weather station, was steadily maintained across the course of the two winter trials until approximately 100ADD. The method became increasingly inaccurate as decomposition stalled but degrees continued to accumulate. This is a common limitation of this method that is reported in the literature where many studies report erroneous PMI predictions once decomposition stalls [24, 53, 117].

The Megyesi et al. [1] protocol performed better at estimating the ADD when temperatures from the BoM weather station were used to calculate the ADD and when the remains were in the early stages of decomposition. This finding is different to the Parsons [53] study where it was reported that the Megyesi et al. [1] method was most inaccurate during the early stages of decay when compared to the other decomposition stages but predictions improved as decomposition progressed. However, Parsons [53] found that once the remains mummified, the method failed to produce an accurate ADD prediction, as was also the case in the present study.

Dautartas [72] reported issues with the scoring model associated with this method and found difficulties 'fitting' remains into the various stages outlined in the protocol, especially once remains mummified as there is no specific score to assign when remains are completely mummified. Difficulties applying a TBS value to remains was found in the current investigation as some changes/stages did not occur in the order of the protocol or did not occur at all, making it difficult to assign an appropriate and accurate TBS to reflect the changes observed. If an inaccurate TBS was assigned to a set of remains this may have impacted on the accuracy of the ADD prediction and, therefore, the validation of predicted ADD against known ADD. As such, the scoring protocol is a limitation of this method.

The Marhoff et al. [2] formula was created by refining the original ADD method by Megyesi et al. [1] to make it specific for use in the Hawkesbury region, NSW, Australia. In Trial 1 and Trial 3, the formula performed best when remains entered the advanced decomposition stage and more accurately estimated the ADD of remains located in the shade than the sun exposed remains. Despite the Marhoff et al. [2] formula being based heavily on the Megyesi et al. [1] protocol, this result is the

opposite to what was found when the Megyesi et al. [1] formula was applied in the present study (see above).

This formula was originally created from a 2013 winter study where it was found that all remains used in that trial reached the advanced decomposition stage [26]. The carcasses had been scored by TBS values equivalent to the advanced decomposition stage for most of the 2013 original trial duration. It is possible the method better estimates the ADD in the latter decay stages, as found in the current study, as it was created from desiccated remains. It is difficult to draw definitive conclusions however, as this formula has not yet been validated in any other study.

When comparing the results of the linear mixed model for both winter trials to determine if the Marhoff et al. [2] method produces more accurate results when obtaining temperatures at the site of the remains or obtaining temperatures from the local weather station (BoM), the method better estimated the ADD when using temperatures recorded by the BoM weather station. This is a positive result as forensic investigators would likely only have access to local weather station data and not data from the post-mortem scene.

The Vass [3] formula performed best overall with positive results observed when the method is applied to estimating PMI when the carcass is in the early decomposition stage. Fewer departures from the x=y line suggests a more linear relationship between the true and calculated PMI. This is consistent with Vass' [3] original study which he proposed this method could only successfully work while remains were in the pre-skeletonisation stages and tissues are soft and pliable. Once the tissues of the carcasses desiccated, hardened and mummified in the current study, the accuracy of the formula was limited.

The Vass [3] formula generally overestimated the known PMI in both Trial 1 and Trial 3 (winter). This is dissimilar to Cockle and Bell's [50] 2015 investigation into the reliability of the Vass formula, where the method underestimated the PMI in colder conditions but overestimated the PMI during warmer weather. Cockle and Bell [50] concluded the Vass [3] formula could not be successfully applied to remains found in Canada. This was likely due to the formula not including all important variables affecting decay rates and these variables may differ from one geographic region to another (e.g. Tennessee to Canada).

Vass notes in the original study that this formula has been applied successfully in regions of the Mid to Eastern United States [3]. He postulated it worked well in these regions because daily humidity levels, vegetation, soil moisture levels and soil type was similar to the Anthropological Research Facility in Tennessee where the method was created, suggesting the method is environmentally specific. Similar humidity levels were found when comparing the average humidity levels of the two winter trials to average humidity levels in winter in Tennessee [118]. Hence, it may be the humidity variable which is contributing to the success of this method when applied under these experimental conditions.

Given the success found when applying the Vass [3] formula and the limited accuracy achieved when applying the Megyesi et al. [1] and Marhoff et al. [2] formulas, it is possible the accuracy of these methods in estimating the PMI is impacted by the means of quantifying decomposition. Both ADD methods make use of a TBS value requiring the researcher to assign a score based on the changes observed according to a predefined scoring protocol. Vass [3] requires the researcher to make a visual assessment of the body and estimate how much soft tissue decomposition has occurred. Accurate PMI estimates found when applying the Vass [3] formula may occur because a body isn't made to 'fit' into a stage of decay but rather a real-time value of soft tissue decomposition is used to estimate the PMI.

Due to the many inaccuracies found when applying the currently published methods, it has been concluded that a new method for PMI estimations of remains deposited in the winter needs to be created based on the data obtained in the present study. This will be presented in Chapter 7.

Chapter Six

6 Result- Testing the Published Methods (The Summer Trials)

6.1 Introduction:

The following section presents the results of the validation of the currently published methods for estimations of PMI for Trial 2 (summer 2014/15) and Trial 4 (summer 2015/16), of the present study. This section will demonstrate:

- ❖ the differences in the recorded climatic data will be analysed to determine if there is a statistically significant difference between the three recording devices. This to determine if there is a difference between weather data recorded at the site and data recorded by the local weather station. If a difference exists, the methods will be evaluated using all temperature recording devices to determine if this impacts on the accuracy of the methods
- ❖ the relationship between
  - o (i) the PMI and TBS (as a representation of decomposition),
  - o (ii) the PMI and the percentage of decomposition,
- ❖ the relationship between the predicted values of ADD and PMI generated by the published protocols, namely
  - o Megyesi et al. [1] (Equation 1),
  - o Marhoff et al. [2] (Equation 2), and
  - o Vass [3] (Equation 3)

and the known PMI and ADD of the remains in the current study.

- ❖ the validity of the Megyesi et al. [1] protocol, the Marhoff et al. [2] formula and the Vass [3] PMI formula in Trial 2 and Trial 4 of the current study will be examined to determine if they can be applied accurately in a Western Sydney region

6.2 Comparison of the climatic data during the winter trials

As each of the three methods [1-3] to be evaluated in the current study rely on temperature to make a PMI determination, temperature data was recorded using three separate devices. Two of these devices were temperature data loggers positioned at the research site on Western Sydney University's Hawkesbury campus. One of these data loggers was located in an area receiving shade for the majority of the day (data logger 1), while the other data logger was positioned in an area receiving direct sunlight during the day (data logger 2). These devices were attached to the cage of one carcass in each of the microclimates. The third medium used to obtain temperature data was the local BoM weather station located at Richmond RAAF base approximately 4.7km from the research site.

6.2.1 Comparison of climatic data: onsite data logger (sun) vs. onsite data logger (shade) for Trial 2

A paired *t*-test was performed to determine the relationship between the two data loggers at the research site, one positioned in the shade (data logger 1) and the other positioned in the sun (data logger 2). It was revealed that the two devices were reporting different temperatures for their respective microclimates and a statistically significant difference was found between the temperatures recorded by data logger 1 in the shade and data logger 2 in the sun for both trials ($p<0.001$).

For data recorded in Trial 2 (summer), it was determined that data logger 2 positioned in the sun was reporting temperatures, on average, 0.80°C higher than the temperatures recorded in the shade. Figure 6-1 demonstrates the relationship between the average daily temperatures recorded by the data loggers in the two microclimates of Trial 2.
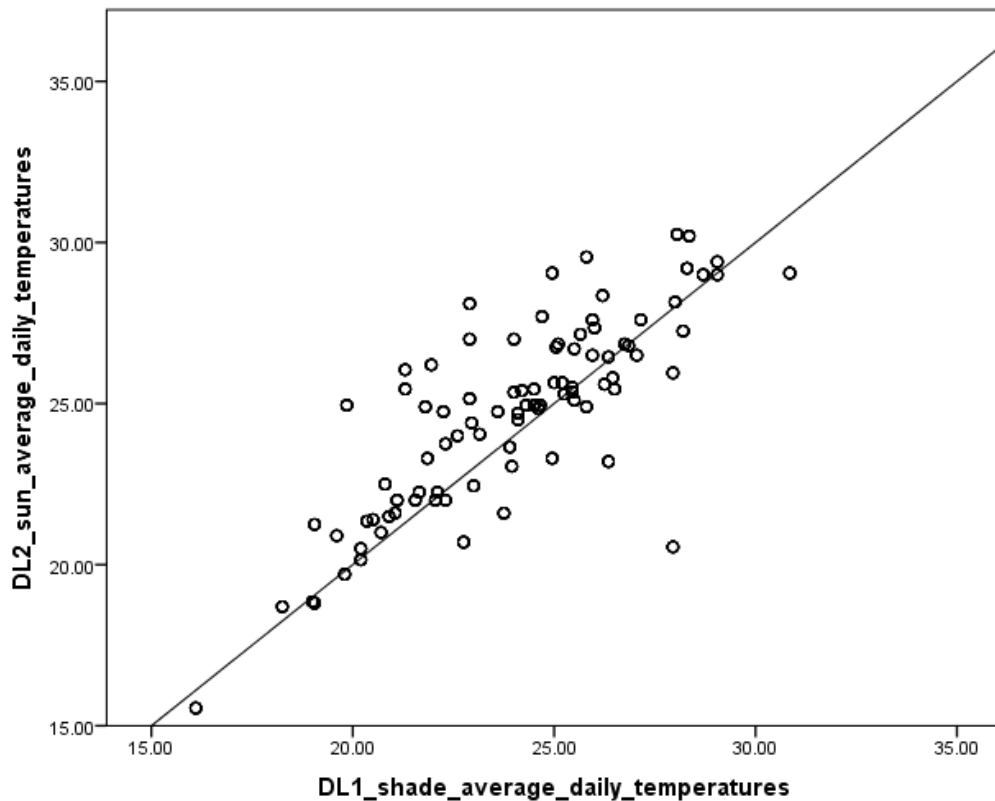
Figure 6-1 Relationship between the average daily temperatures reported by the data loggers at the research site located in the shade (DL1) and in the sun (DL2) in Trial 2. All units are in degrees Celsius (°C).

6.2.2 Comparison of climatic data: onsite data logger (sun) vs. onsite data logger (shade) for Trial 4

A paired *t*-test was performed to determine the relationship between the two data loggers at the research site, one positioned in the shade (data logger 1) and the other positioned in the sun (data logger 2). It was revealed that the two devices were reporting different temperatures for their respective microclimates and a statistically significant difference was found between the temperatures recorded by data logger 1 in the shade and data logger 2 in the sun for both trials ($p<0.001$).

For data recorded in Trial 4 (summer), it was determined that data logger 2 positioned in the sun was reporting temperatures that were, on average, 2.15°C higher than the temperatures recorded in the shade. Figure 6-2 shows the relationship between the temperatures recorded by the data loggers in the two microclimates of Trial 4.
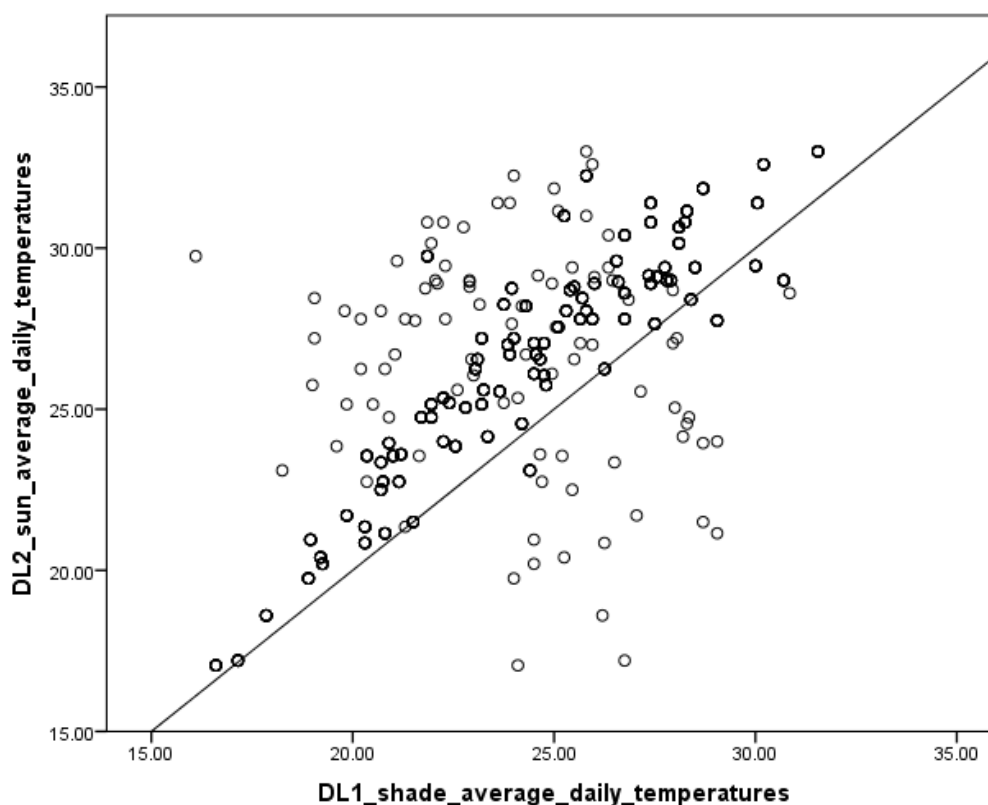
Figure 6-2 Relationship between the average daily temperatures reported by the data loggers at the research site located in the shade (DL1) and in the sun (DL2) in Trial 4. All units are in degrees Celsius (°C).

6.2.3 Comparison of climatic data: BoM weather station vs. onsite data logger (shade)

Figure 6-3 and Figure 6-4 demonstrate the relationship between the temperatures recorded by the BoM weather station and the temperatures recorded by the data logger at the research site positioned in the shade (data logger 1) for Trials 2 and 4. There was a statistically significant difference between the temperatures recorded at the site for both trials and the temperatures reported by the BoM weather station (Trial 2; $p<0.001$, Trial 4; $p<0.001$). The data revealed that temperatures recorded by the BoM weather station in Trial 2 were, on average, 0.56°C higher than the temperatures reported for data logger 1. For Trial 4, data logger 1 (shade) reported temperatures 0.80°C higher than the temperatures reported by the BoM weather station.
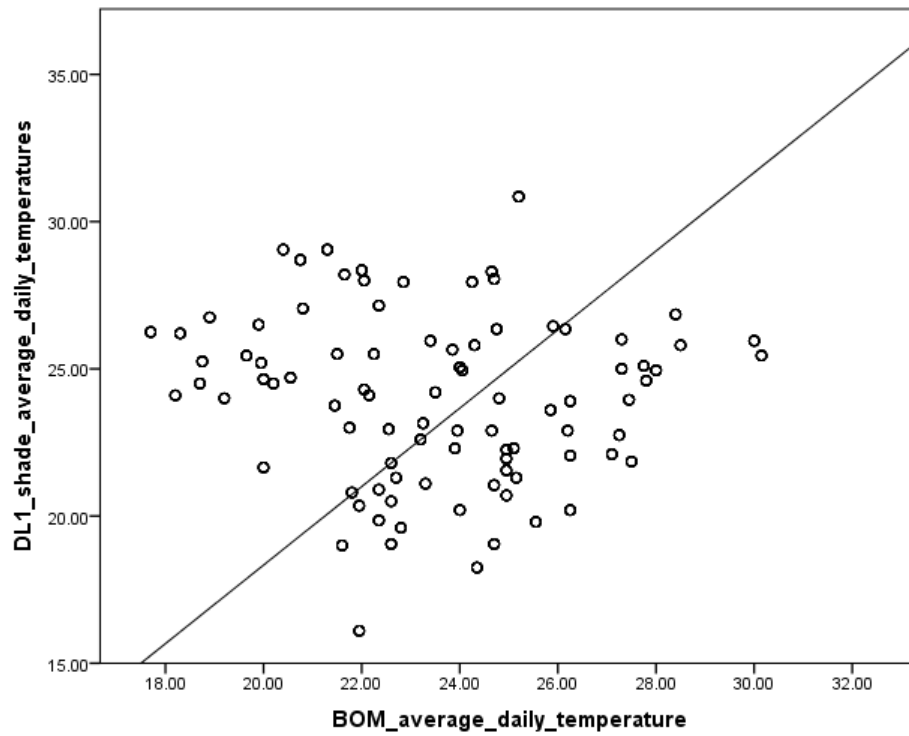
Figure 6-3 Relationship between the average daily temperatures reported by the data logger at the research site located in the shade and the temperatures reported by the BoM weather station in Trial 2. All units are in degrees Celsius (°C).
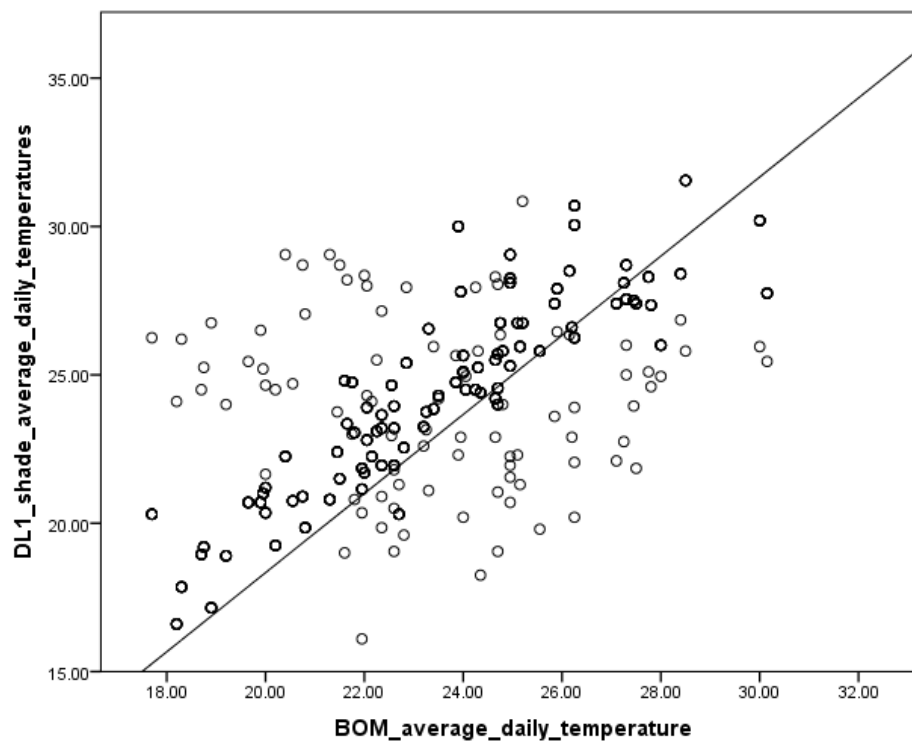
Figure 6-4 Relationship between the average daily temperatures reported by the data logger at the research site located in the shade and the temperatures reported by the BoM weather station in Trial 4. All units are in degrees Celsius (°C).

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6.2.4 Comparison of climatic data: BoM weather station vs. onsite data logger (sun)

A paired *t*-test was also performed to determine the relationship between the temperatures reported by the BoM weather station at Richmond RAAF base and the temperatures recorded at the research site for the data logger positioned in the sun for Trial 2 and Trial 4. It was revealed there was a statistically significant difference between the temperatures recorded at the research site and the BoM weather station during both summer trials ($p<0.001$). The temperature data recorded by the data logger in the sun (data logger 2) was, on average, 1.36°C higher than the temperatures reported by the BoM weather station for the area in Trial 2 and 2.95°C higher than temperatures recorded by the BoM weather station for Trial 4. Figure 6-5 and Figure 6-6 shows the relationship between the temperatures recorded by the data logger in the sun (data logger 2) and the BoM weather station for the two summer trials.
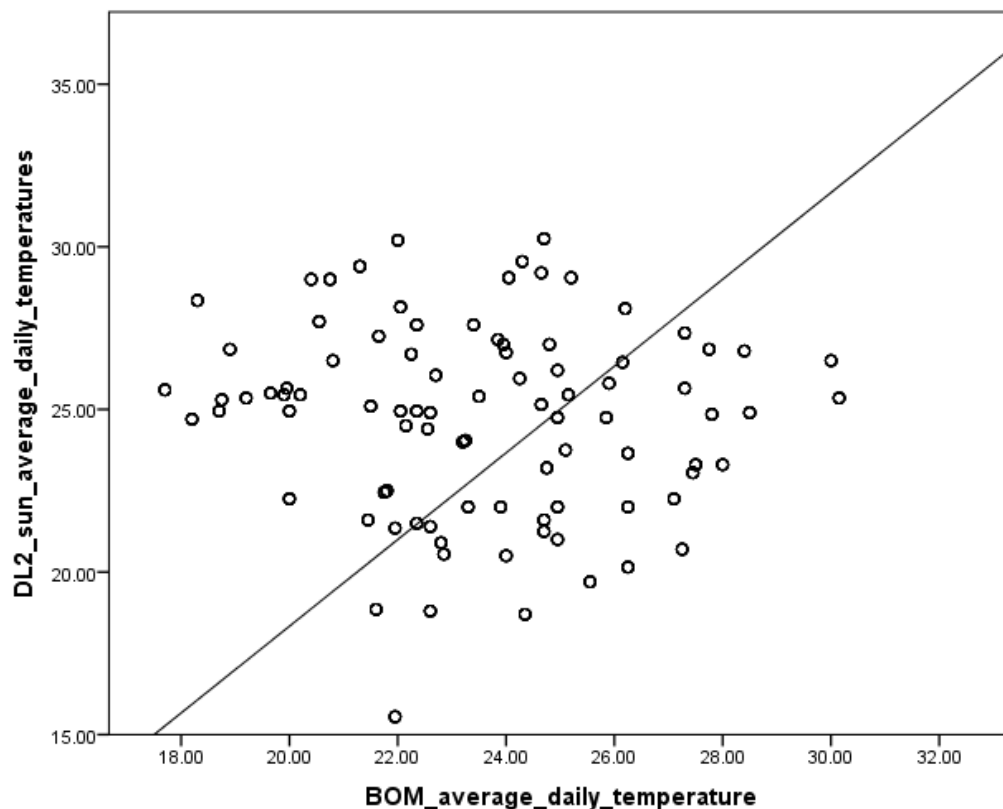
Figure 6-5 Relationship between the average daily temperatures reported by the data logger at the research site located in the sun and the temperatures reported by the BoM weather station in Trial 2. All units are in degrees Celsius (°C).
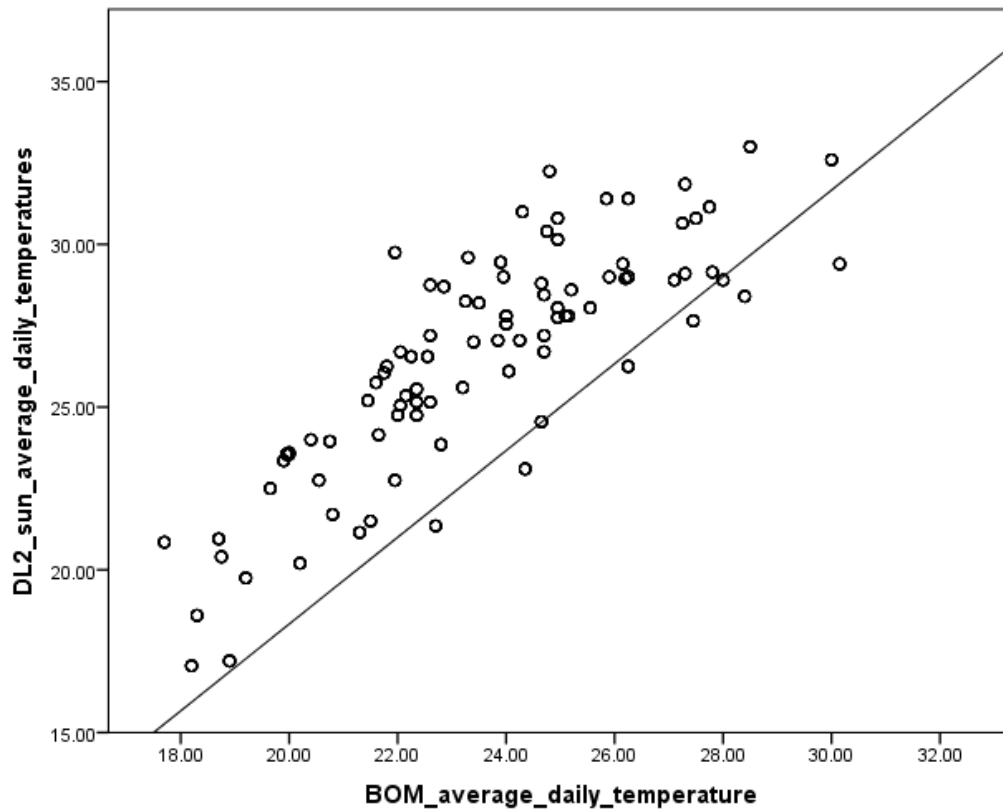
Figure 6-6 Relationship between the average daily temperatures reported by the data logger at the research site located in the sun and the temperatures reported by the BoM weather station in Trial 4. All units are in degrees Celsius (°C).

6.2.5 Discussion

As observed during the winter trials (Chapter 5), statistically significant differences were found when evaluating the relationships between the three temperature apparatus. It was therefore concluded that the analyses to follow would be performed using the temperature data from all three sources. The temperatures recorded by the two respective data loggers (data logger 1 and data logger 2) would subsequently be used as the temperature variable in PMI calculations of the remains in the individual microclimates (sun and shade). That is, temperatures recorded by data logger 1 would be used when estimating the PMI of the carcasses positioned in the shade and temperatures recorded by data logger 2 would be used in all analyses of the sun exposed remains. The data recorded by the BoM weather station at Richmond RAAF base would also be used to conduct a separate analysis to determine if the Megyesi et al. [1] protocol, the Marhoff et al. [2] formula and the Vass formula [3] could better estimate the PMI using the local weather station data, as this data is likely to be the

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only source of weather data available in a forensic case, or, if the methods better estimate the PMI when data is recorded at the 'discovery' site.

6.3   Validating the published methods for PMI estimates

To determine if the methods were accurately working in a temperate Australian environment, the estimated ADD and PMI found using the published equations were plotted against the known ADD and PMI for the respective equations for Trial 2 (Figure 6-8, Figure 6-9, Figure 6-13, Figure 6-14 and Figure 6-18) and Trial 4 (Figure 6-11, Figure 6-12, Figure 6-15, Figure 6-16 and Figure 6-20). Visually, for each set of remains and for each protocol tested, there were strong departures from the x=y line, therefore, a linear relationship did not exist between the predicted and known values.

Linear mixed modelling was performed to examine the relationship between the known ADD and PMI of the study and the estimated ADD and PMI found using the predictive equations by Megyesi et al. [1], Marhoff et al. [2] and Vass' PMI formula [3]. The data was computed without the intercept, using the syntax function in SPSS, to determine if the intercept was equal to 1. If the intercept was equal to 1 there was a linear trend between the two variables [pers. comm. Paul Fahey, Western Sydney University].

Each of the linear mixed models showed the PMI/ADD values generated by the predictive methods to be highly statistically different ($p<0.001$) to the known ADD and PMI values in the present study. This means there was a difference between the PMI/ADD that the methods were estimating and the known values of PMI/ADD, which suggests that these equations are ineffective for estimating time since death under the current experimental conditions.

To confirm the result of the linear mixed models, linear regression was performed. If an adjusted r^2 value of 0.80 or higher was achieved (80% accuracy), the published methods were considered to be reasonable predictors of the true ADD or PMI. An adjusted r^2 value of 0.80 was used in order to be comparable with previous studies in the literature [1, 117].

The results of the analyses for the Megyesi et al. [1], the Marhoff et al. [2], and the Vass [3] formulas are presented individually for Trial 2 and Trial 4 (summer) below.

6.4 Validating the Megyesi et al. ADD method- Trial 2

6.4.1 Trial 2 (summer) body scored using TBS

Figure 6-7 depicts the relationship between the TBS assigned using the Megyesi et al. [1] scoring protocol for all eight carcasses of Trial 2 (summer) of the present study against the known PMI.

Initially, as seen in Figure 6-7, decomposition progressed rapidly resulting remains being scored TBS values of 15-18 between Day 3 and Day 5. As the decomposition process continued, the TBS continued to increase each day in the PMI. From approximately Day 40, when the remains were in the advanced decomposition stage, decay rates began to slow dramatically and stall, resulting in the same TBS value being assigned multiple times. This can be viewed as a series of plateaus between Days 40 and 60 (as indicated by the purple arrow) and again at Days 65 to 90 (as indicated by the orange arrow) in Figure 6-7. While time transpired, decomposition did not progress as equally.

Between Days 40 to 60 approximately 142.6mm of rain fell during this time which may have hydrated the remains, causing decomposition process to once again become active. This resulted in an increase in the TBS for all sets of remains with the exception of Pig 4, however, as mentioned above, the remains once again entered a period of stasis where while time transpired (as indicated by the orange arrow), no further decomposition changes occurred.
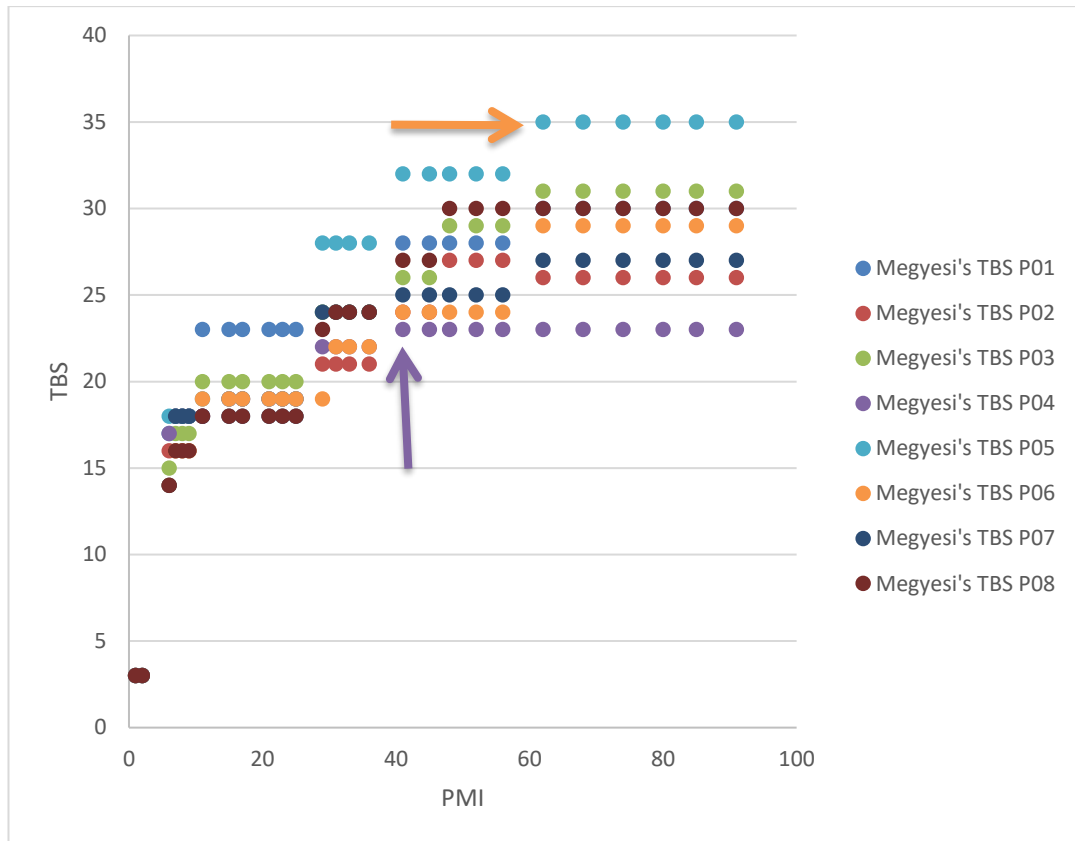
Figure 6-7 Scatterplot of TBS vs. PMI (in days) for all carcasses (Pigs 1-8) to demonstrate the relationship between transpired time and the decomposition of remains for Trial 2. Purple arrow indicates a plateau between Days 40 and 60; orange arrow indicates beginning of plateau at Day 65 to the end of the trial at Day 90.

6.4.2 Using temperature data recorded at the research site

Although Figure 6-7 demonstrates that a similar pattern of decay was observed for all carcasses contrary to the microclimate they were located in, for ease of reporting the results, the graphs were separated into the shaded and sun exposed microclimates.

Figure 6-8 depicts the predicted ADD against the known ADD of remains, as recorded by the onsite data loggers, located in the sun exposed (Figure 6-8a) and shaded (Figure 6-8b) microclimates. It can be observed from the graph below that the variation between the known and predicted PMI greatly increased as the trial progressed and at no time point was the predicted and known values comparable. Pig 5 (shade) is clearly the outlier (yellow line) and when plotted it can be observed that the predicted ADD for this carcass is dramatically overestimated when compared to the true ADD value and the other carcasses in this trial. Improvements in the validation results may be found if this particular carcass was not included in the testing.

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Due to software limitations, the line of best fit for both the shaded remains and the sun exposed remains appears on both Figure 6-8a and Figure 6-8b. Therefore, the line of best fit for shaded remains is indicated by the purple arrow and the line of best fit for sun exposed remains is indicated by the orange arrow.

6.4.2.1 Sun exposed microclimate

The predicted ADD values found using the Megyesi et al. [1] method for carcasses in the sun exposed microclimate were compared to the known ADD recorded by the data logger in the sun at the research site (Figure 6-8a).

The linear mixed modelling demonstrated a statistically significant difference between the predicted ADD and the known ADD recorded during Trial 2 using the Megyesi et al. [1] predictive method on sun exposed remains, with the fixed effect being 0.115 (t (211.8) =-9.83, $p<$ 0.000). These results suggest that, on average, the formula overestimated the known ADD during Trial 2. Hence, the Megyesi et al. [1] method was predicting the known ADD of the sun exposed remains found in the Hawkesbury region to be 0.115 times greater than the known ADD value.

Linear regression analysis was performed to determine how well the predicted model was estimating the ADD of the remains in the present study. Regression analysis yielded an r^2 value of 0.532, suggesting the method was a poor predictor of the true ADD of sun exposed remains in the current study when using temperatures obtained from the 'discovery' site.

6.4.2.2 Shaded microclimate

The predicted ADD values found using the Megyesi et al. [1] formula for carcasses in the shaded microclimate were compared to the known ADD recorded by the data logger in the shade at the research site.

The linear mixed modelling demonstrated a statistically significant difference between the predicted ADD and the known ADD recorded during Trial 2 using the Megyesi et al. [1] method on shaded remains. The fixed effect for shaded remains, expressed as the slope, increased from the intercept (0.115; as recorded for sun exposed remains) by 0.278, resulting in a fixed effect of 0.394 (t (211.6) =7.91, $p<$0.000). These results suggest that, on average, the formula overestimated the known ADD during Trial 2. Hence, the ADD predicted by the Megyesi et al. [1]

method was 0.394 times greater than the known ADD recoded by the onsite data loggers.

Linear regression analysis was performed to determine how well the predicted model was estimating the ADD of the remains in the present study. Regression analysis yielded an r^2 value of 0.334, suggesting the method was a poor predictor of the ADD of shaded remains in the current study when using temperatures obtained from the 'discovery' site.
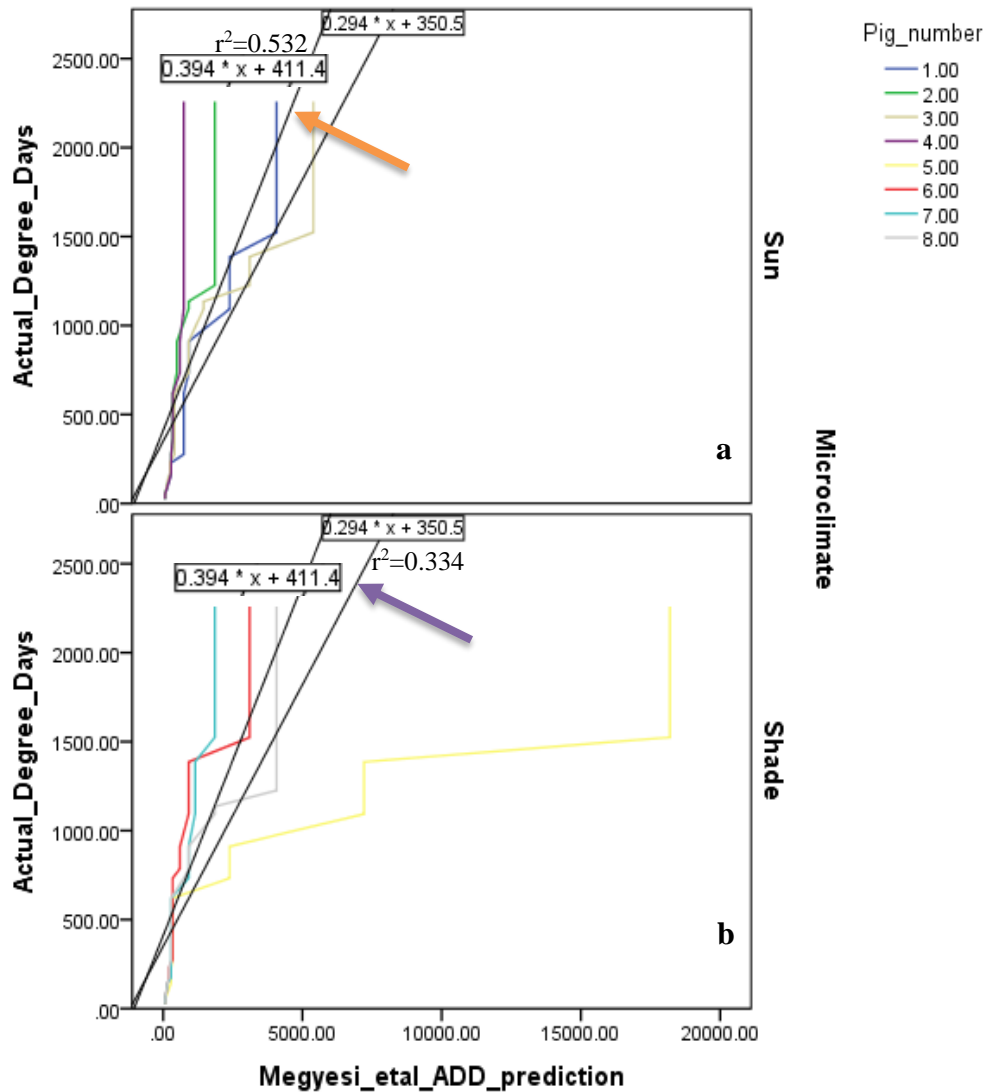
Figure 6-8 The Megyesi et al. [1] prediction of ADD vs. the known ADD of all carcasses (n=8), where 0.394*x+411.4 is the line of best fit for the carcasses located in the sun (orange arrow) and 0.294*x+350.5 is the line of best fit for carcasses located in the shade (purple arrow) for Trial 2 summer 2014/15. Units in 'days °C'.

6.4.3 Using temperature data collected from the BoM weather station

Figure 6-9 depicts the predicted ADD against the known ADD, as recorded by the BoM weather station, for remains located in the sun exposed (Figure 6-9a) and shaded (Figure 6-9b) microclimates. One again, it can be observed in Figure 6-9a-b that a close relationship between the predicted and known ADD was not observed at any time point in Trial 2 when employing either the temperatures at the research site or temperatures obtained from the BoM weather station.

Due to software limitations, the line of best fit for both the shaded remains and the sun exposed remains appears on both Figure 6-9a and Figure 6-9b. Therefore, the line of best fit for shaded remains is indicated by the purple arrow and the line of best fit for sun exposed remains is indicated by the orange arrow.

6.4.3.1 Sun exposed microclimate

The predicted ADD values found using the Megyesi et al. [1] method for carcasses in the sun exposed microclimate were compared to the known ADD recorded by the BoM weather station, using linear mixed modelling.

The results of the linear mixed modelling showed there was a significant difference between the predicted ADD and the known ADD recorded during Trial 2 using the Megyesi et al. [1] predictive method on the sun exposed remains, with the fixed effect being 0.110 (t (211.8) =9.86, p<0.000). The results of the linear mixed model suggest that, on average, the formula overestimated the known ADD of the shaded remains during Trial 2 and the ADD predicted by the Megyesi et al. [1] formula was 0.110 times greater than the known ADD values, when temperatures were obtained from the BoM weather station.

Linear regression analysis was performed to determine how well the predicted model was estimating the ADD of the remains in the present study. Regression analysis yielded an r^2 value of 0.533, suggesting the method was a poor predictor of the true ADD of sun exposed remains in the current study when using temperatures obtained from the BoM weather station

6.4.3.2 Shaded microclimate

The predicted ADD values found using the Megyesi et al. [1] method for carcasses in the shaded microclimate were compared to the known ADD recorded by the local BoM weather station, using linear mixed modelling.

The results of the linear mixed modelling showed there was a statistically significant difference between the predicted ADD and the known ADD recorded during Trial 2 using the Megyesi et al. [1] method on shaded remains. The fixed effect for shaded remains, expressed as the slope, increased from the intercept (0.110; as recorded for sun exposed remains) by 0.115, resulting in a fixed effect of 0.266 (t (11.6) =7.94, $p<0.000$). The results of the linear mixed model suggest that, on average, the formula overestimated the true ADD of the shaded remains during Trial 2. Hence, the Megyesi et al. [1] formula predicted the ADD to be 0.266 times greater than the known ADD values, when temperatures were obtained from the BoM weather station.

Linear regression analysis was performed to determine how well the predicted model was estimating the ADD of the remains in the present study. Regression analysis yielded an r^2 value of 0.335, suggesting the method was a poor predictor of the true ADD of shaded remains in the current study when using temperatures obtained from the BoM weather station.
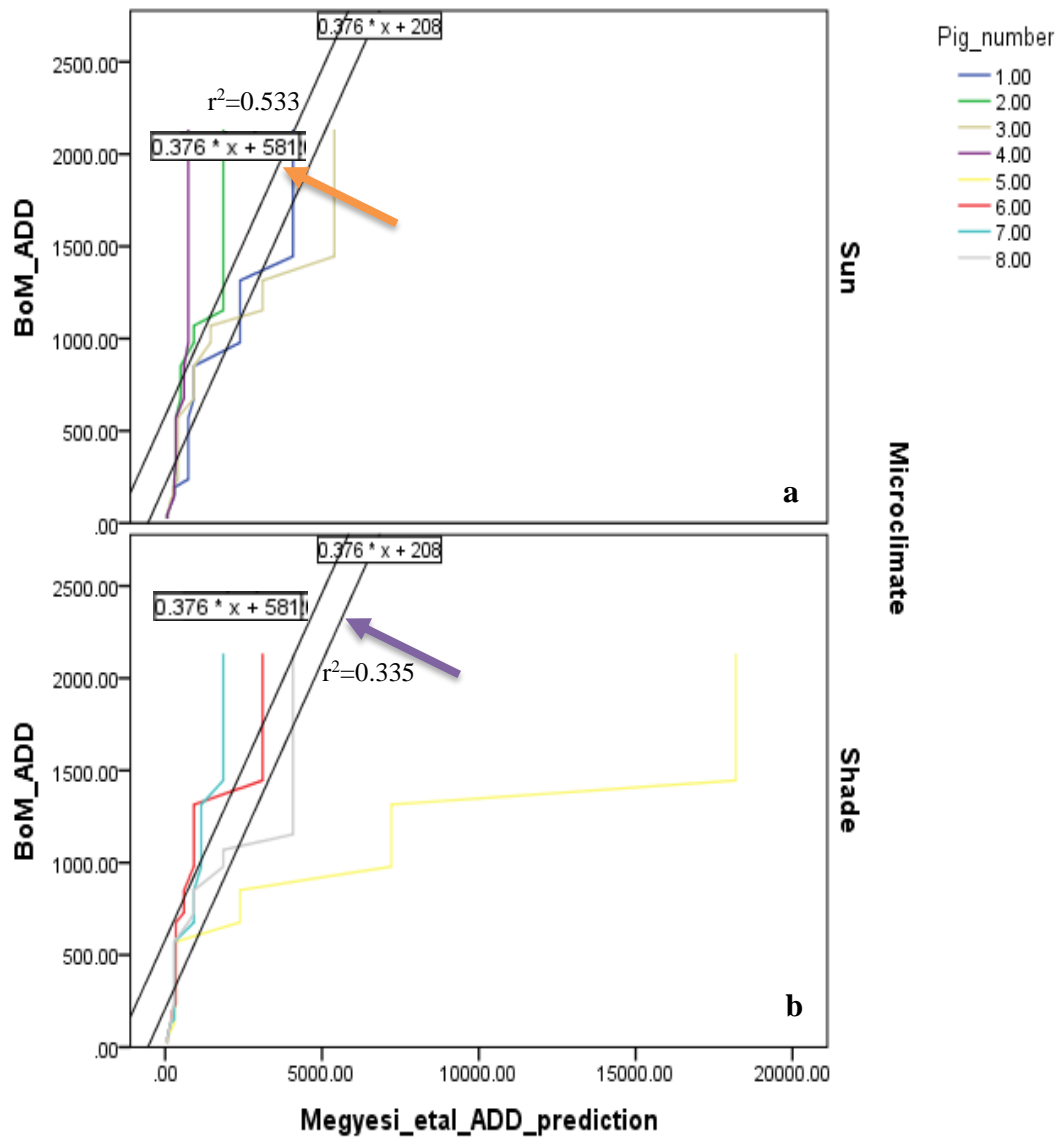
Figure 6-9 The Megyesi et al. [1] prediction of ADD vs. the known ADD of all carcasses (n=8), where 0.376*x+581 is the line of best fit for the sun carcasses (orange arrow) and 0.376*x+208 is the line of best fit for shaded carcasses (purple arrow) for Trial 2 summer 2014/15. Units in 'days °C'.

6.5   Validating the Megyesi et al. ADD method- Trial 4

6.5.1   Trial 4 (summer) body scored using TBS

Figure 6-10 depicts the relationship between the TBS assigned, using the Megyesi et al. [1] scoring protocol, for all eight carcasses of Trial 4 (summer) of the present study against the known PMI.

A curvilinear pattern is observed in Figure 6-10 when the TBS is plotted against the known PMI for Trial 4 (Figure 6-10). Initially in the decomposition process,

decomposition occurred rapidly and the TBS values assigned to remains were increasing on each sampling as gross morphological changes were observed. While decomposition progressed quickly, it soon slowed between Days 10 and 30 (as indicated by the blue arrow), where few decomposition changes were observed, resulting in the same TBS value being assigned multiple times to the remains. At this time, significant biomass had been lost and the carcasses exhibited extensive bone exposure (see Chapter 4). Due to the advanced rate of soft tissue decay early in the post-mortem period, few changes were left to occur as time transpired. Similar to Trial 2, this resulted in the extensive plateaus outlined in Figure 6-10 between Days 30 and 70 (as indicated by the orange arrow) and again between Days 70 and 90 (as indicated by the purple arrow), which suggests that although time was continuing to pass; few taphonomic changes were taking place. Decomposition slowed less frequently in Trial 4 but remained in a fixed state of decay for longer. This is in contrast to Trial 2 where decomposition was observed to stall many times over the length of the trial but for shorter periods of time.
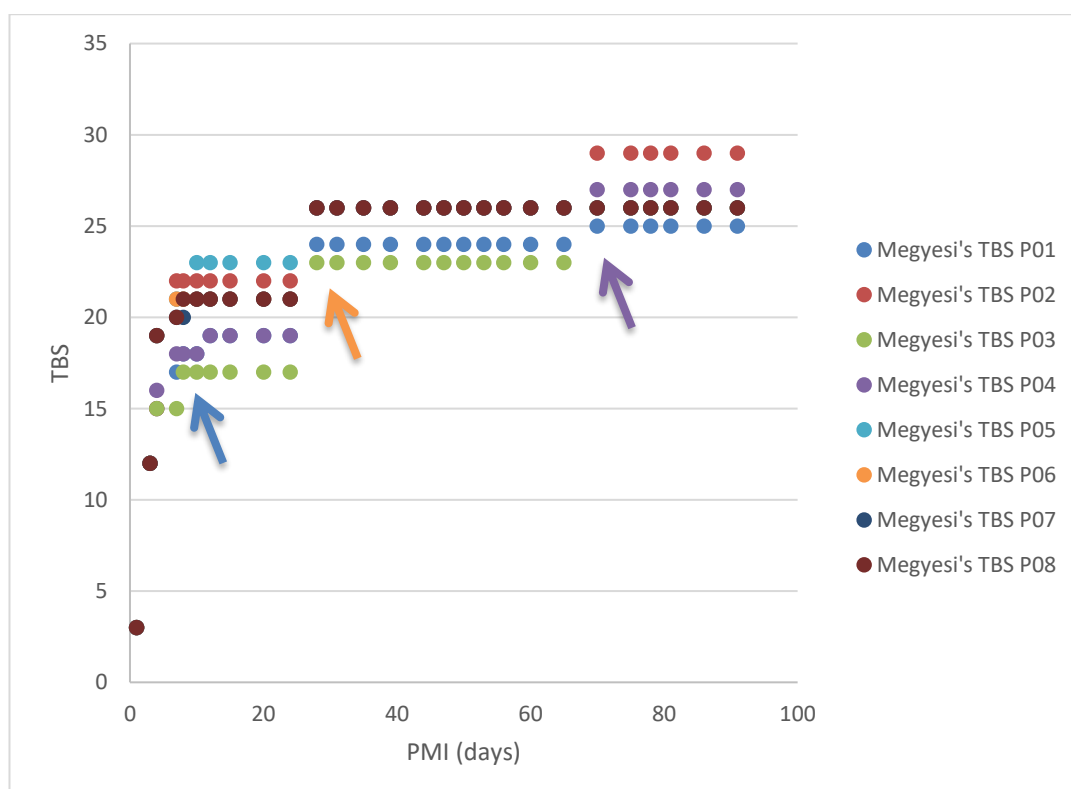
Figure 6-10 Scatterplot of TBS vs. PMI (in days) for all 8 carcasses (Pigs 1-8) to demonstrate the relationship between transpired time and the decomposition of remains in Trial 4. Blue arrow indicates a plateau between Day 10 and 30, orange arrow indicates plateau from Day 30 and 70, purple arrow indicates plateau between Day 70 and 90.

6.5.2 Using temperature data collected at the research site

Although Figure 6-10 demonstrates that a similar pattern of decay was observed for all carcasses contrary to the microclimate they were located in, for ease of reporting the results the graphs will now be separated into the shaded and sun exposed microclimates.

Figure 6-11 depicts the predicted ADD against the known ADD of remains, as recorded by the onsite data loggers, located in the shaded (Figure 6-11a) and sun exposed (Figure 6-11b) microclimates. It can be observed in Figure 6-11, that a close relationship between the known and estimated PMI only existed between carcasses until approximately 900ADD before a separation between carcasses and variables was observed.

Due to software limitations, the line of best fit for both the shaded remains and the sun exposed remains appears on both Figure 6-11a and Figure 6-11b. Therefore, the line of best fit for shaded remains is indicated by the purple arrow and the line of best fit for sun exposed remains is indicated by the orange arrow.

6.5.2.1 Shaded microclimate

The predicted ADD values found using the Megyesi et al. [1] formula for carcasses in the shaded microclimate were compared to the known ADD recorded by the data logger in the shade at the research site.

The linear mixed modelling demonstrated a statistically significant difference between the predicted ADD and the actual ADD recorded during Trial 4 using the Megyesi et al. [1] method on shaded remains. The fixed effect for shaded remains, expressed as the slope, decreased from the intercept (1.16; as recorded for sun exposed remains) by 0.317, resulting in a fixed effect of 0.845 (t (209.2) = -3.43, p<0.001). These results suggest that, on average, the formula overestimated the true ADD during Trial 4 and the ADD estimated by the Megyesi et al. [1] formula was 0.845 times greater than the known ADD recorded by the onsite data logger.

Linear regression analysis was performed to determine how well the predicted model was estimating the ADD of the remains in the present study. Regression analysis yielded an r^2 value of 0.619, suggesting the method was a poor predictor of the true

ADD of shaded remains in the current study when using temperatures obtained from the 'discovery' site.

6.5.2.2 Sun exposed microclimate

The predicted ADD values found using the Megyesi et al. [1] formula for carcasses in the sun exposed microclimate were compared to the known ADD recorded by the data logger in the sun at the research site, using linear mixed modelling.

The linear mixed modelling demonstrated a statistically significant difference between the predicted ADD and the known ADD recorded during Trial 4 when using the Megyesi et al. [1] method on sun exposed remains, with the fixed effect being 1.16 (t (205.8) = 15.7, p<0.000). This suggests that, on average, the formula underestimated the known ADD during Trial 4 and the known ADD of the sun exposed remains found in the Hawkesbury region was actually 1.16 times greater than the ADD predicted by the Megyesi et al. [1] method.

Linear regression analysis was performed to determine how well the predicted model was estimating the ADD of the remains in the present study. Regression analysis yielded an r^2 value of 0.654, suggesting the method was a poor predictor of the true ADD of sun exposed remains in the current study when using temperatures obtained from the 'discovery' site.
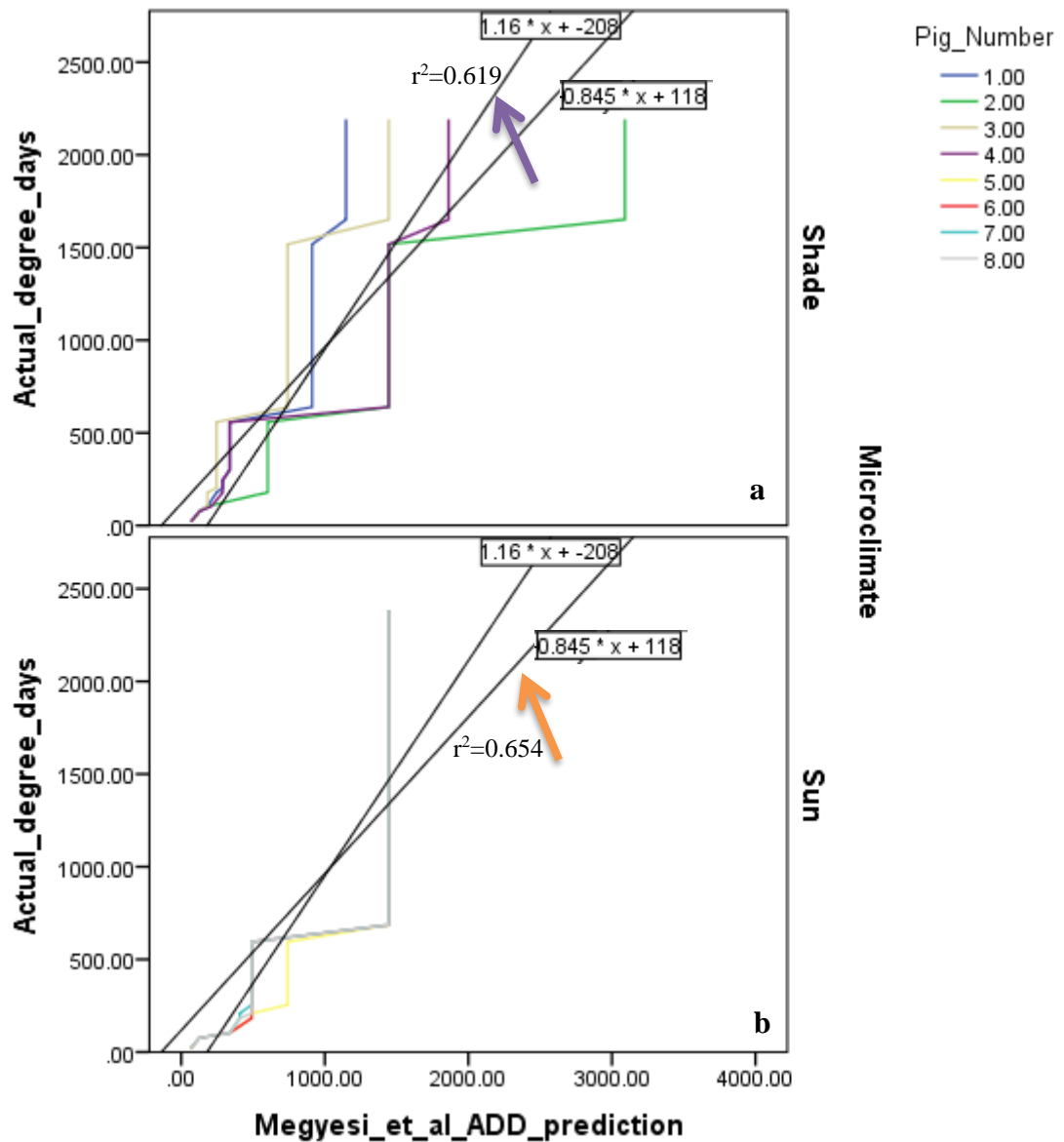
Figure 6-11 The Megyesi et al. [1] prediction of ADD vs. the actual ADD of all carcasses (n=8), where 1.16*x+-208 is the line of best fit for carcasses located in the shade (purple arrow) and 0.845*x+118 is the line of best fit for the carcasses located in the sun (orange arrow) for Trial 4 summer 2015/16. Units in 'days °C'.

6.5.3 Using temperature data collected from the local BoM weather station

Figure 6-12 depicts the predicted ADD against the known ADD, as recorded by the local BoM weather station, for remains located in the shaded (Figure 6-12a) and sun exposed (Figure 6-12b) microclimates. When temperatures from the BoM weather station are employed in the estimate of ADD, a similar pattern is observed to when temperatures at the research site are used. This result suggests that the use of temperature obtained by either data source will produce a similar ADD estimate.

Due to software limitations, the line of best fit for both the shaded remains and the sun exposed remains appears on both Figure 6-12a and Figure 6-12b. Therefore, the line of best fit for shaded remains is indicated by the purple arrow and the line of best fit for sun exposed remains is indicated by the orange arrow.

6.5.3.1   Shaded microclimate

The predicted ADD values found using the Megyesi et al. [1] method for carcasses in the shaded microclimate were compared to the known ADD recorded by the local BoM weather station.

The results of the linear mixed modelling showed there was a statistically significant difference between the predicted ADD and the actual ADD recorded during Trial 4 using the Megyesi et al. [1] method on shaded remains. The fixed effect for shaded remains, expressed as the slope, decreased from the intercept (1.05; as recorded for sun exposed remains) by 0.219, resulting in a fixed effect of 0.798 (t (208.8) =-0.219, $p<0.010$). The results of the linear mixed model suggest that, on average, the formula overestimated the true ADD of the shaded remains during Trial 4. Hence, the predicted ADD using the Megyesi et al. [1] method was 0.798 times greater than the known ADD, when temperatures were recorded at the local BoM weather station.

Linear regression analysis was performed to determine how well the predicted model was estimating the ADD of the remains in the present study. Regression analysis yielded an r^2 value of 0.620, suggesting the method was a poor predictor of the true ADD of shaded remains in the current study when using temperatures obtained from the local BoM weather station.

6.5.3.2   Sun exposed microclimate

The predicted ADD values found using the Megyesi et al. [1] formula for carcasses in the sun exposed microclimate were compared to the known ADD recorded by the local BoM weather station, using linear mixed modelling.

The results of the linear mixed modelling showed there was a statistically significant difference between the predicted ADD and the known ADD recorded during Trial 4 using the Megyesi et al. [1] predictive method on the sun exposed remains, with the fixed effect being 1.05 (t (205.8) =15.5, $p<0.000$). The results of the linear mixed model suggest that, on average, the formula underestimated the true ADD of the sun

exposed remains during Trial 4. Hence, the known ADD of sun exposed carcasses in this trial was 1.05 times greater than the predicted ADD using the Megyesi et al. [1] formula, when temperatures were recorded at the local BoM weather station.

Linear regression analysis was performed to determine how well the predicted model was estimating the ADD of the remains in the present study. Regression analysis yielded an r^2 value of 0.668, suggesting the method was a poor predictor of the true ADD of sun exposed remains in the current study when using temperatures obtained from the BoM weather station.
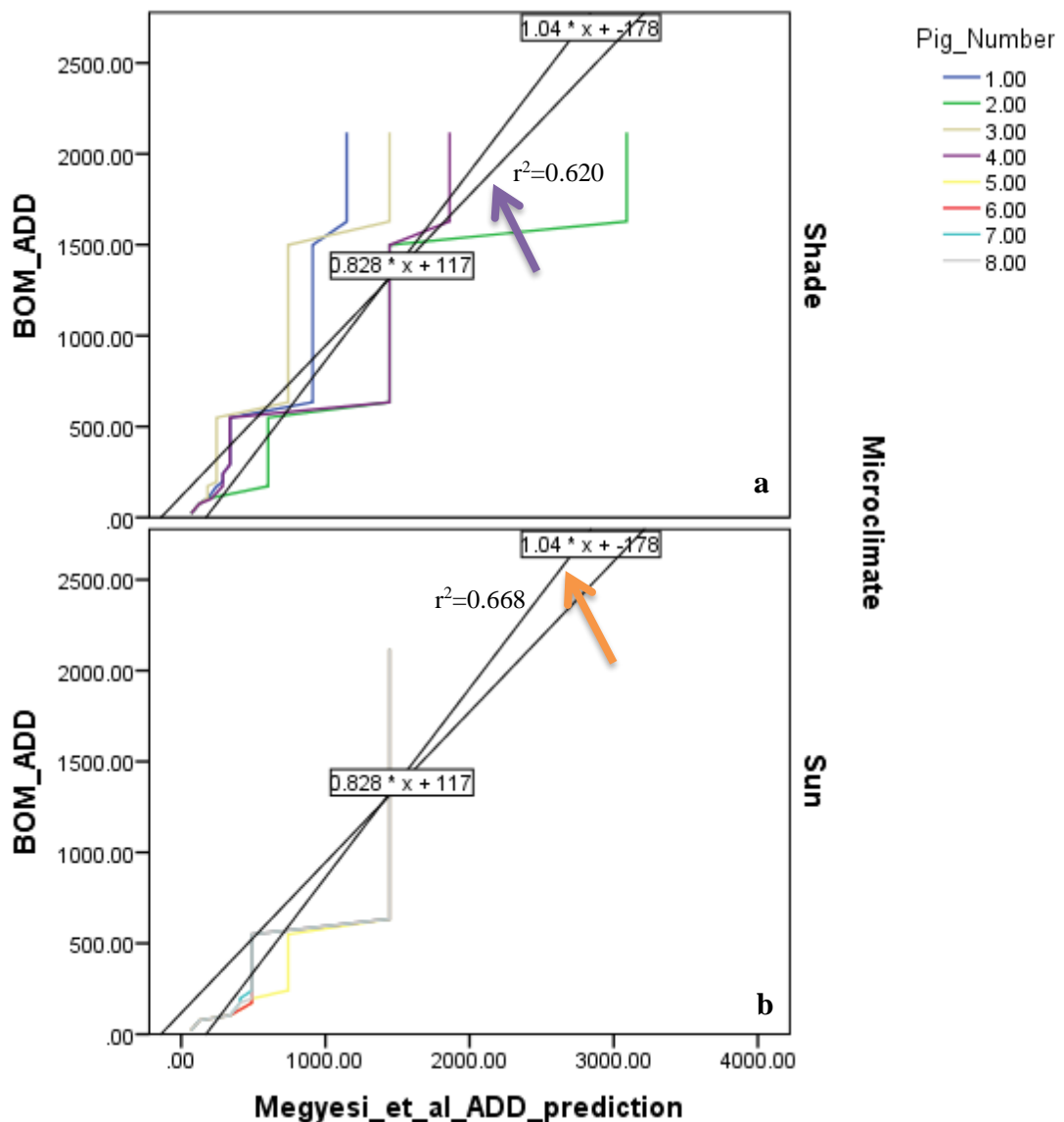
Figure 6-12 The Megyesi et al. [1] prediction of ADD vs. the actual ADD of all carcasses (n=8), where 0.828*x+117 is the line of best fit for carcasses located in the shade (purple arrow) and 1.04*x+-178 is the line of best fit for the carcasses located in the sun (orange arrow) for Trial 4 summer 2015/16. Units in 'days °C'.

6.5.4 Summary

The results of the validation of the Megyesi et al. [1] method for determining PMI indicate this method is not appropriate for accurately determining the PMI in a Western Sydney summer climate. In Trial 2, the variation between the predicted and known ADD increased dramatically over the course of the trial and at no time point could this method estimate the ADD correctly. Although a similar ADD was estimated for each carcass, it should be noted that this was not correctly determined. This result was different to Trial 4, where it was observed (using both weather station data and data logger temperatures) that a close relationship between the predicted and known ADD was present up until approximately 900ADD.

Table 6-1 and Table 6-2 numerically summarises the application of the Megyesi et al. [1] method at randomly selected 'discovery' dates from Trial 2 and Trial 4, respectively. Results are reported from a single carcass (Pig 1; Trial 2 (shade), Trial 4 (sun)), with complete results reported in Appendix I and Appendix O.

The summary tables confirm the pattern observed in Figure 6-8, Figure 6-9, Figure 6-11 and Figure 6-12 and demonstrates the overall inaccuracy of the ADD method when it was applied to decomposed carcasses in the two summer trials, Trial 2 and Trial 4. It can be observed in Table 6-1 that the method was accurate only up to Day 5, however, Table 6-2 shows the method estimated the PMI to within 3 days up until Day 40 of the trial.

Table 6-1 Summary table comparing the predicted ADD with the known/true ADD for Pig 1 (shade) in Trial 2[*].

PMI (in days)	Predicted ADD[*]:	Known ADD: Data Logger	Predicted ADD[*]: BoM	Known ADD: BoM
5	244.34 (9.92 days)	144.80	244.34 (10.42 days)	127.15
10	737.90 (29.95 days)	276.45	737.90 (31.48 days)	236.75
15	737.90 (29.95 days)	392.75	737.90 (31.48 days)	352.65
40	2387.81 (96.91 days)	1093.40	2387.81 (101.86 days)	978.65
90	4073.80 (165.33 days)	2258.25	4073.80 (173.79 days)	2133.27

[*] ADD expressed as days °C. The ADD has been converted to days and appears in the brackets within the table.

Table 6-2 Summary table comparing the predicted ADD with the known/true ADD for Pig 1 (sun) in Trial 4[*].

Day in PMI	Predicted ADD[*]	Known ADD: Data Logger	Predicted ADD[*]: BoM	Known ADD: BoM
5	181.97 (7.51 days)	128.40	181.97 (7.73 days)	125.35
10	288.40 (11.9 days)	245.70	288.40 (12.25 days)	240.40
15	338.34 (13.9 days)	369.75	338.34 (14.37 days)	366.10
40	912.01 (37.6 days)	917.55	912.01 (38.74 days)	908.14
90	1148.15 (47.44 days)	2191.80	1148.15 (48.77 days)	2117.55

[*] ADD expressed as days °C. The ADD has been converted to days and appears in the brackets within the table.

On average, the published method overestimated the ADD in the two summer trials by approximately 0.592 times the true ADD value, using both temperatures obtained at the research site and the local weather station. Applying this method in Trial 4 produced better estimates of the ADD when compared to the results found in Trial 2, however, accuracy is still below an accepted level of 80% and the method was only accurate until approximate Day 40 during Trial 4.

The results of the validation of the Megyesi et al. [1] method will be compared to the other published methods at the conclusion of this chapter.

6.6   Validating the Marhoff et al. formula - Trial 2

6.6.1   Using temperature data recorded at the research site

Figure 6-13 depicts the predicted ADD found using the Marhoff et al. [2] formula against the known ADD of remains, as recorded by the onsite data loggers, located in the sun exposed (Figure 6-13a) and shaded (Figure 6-13b) microclimates. Both microclimates exhibited similarities in the predicted and known ADD at approximately 700ADD and 1000ADD. At all other time points the variation between the true and estimated ADD for each carcass was large and the difference between these values increased as time progressed.

Due to software limitations, the line of best fit for both the shaded remains and the sun exposed remains appears on both Figure 6-13a and Figure 6-13b. Therefore, the line of best fit for shaded remains is indicated by the purple arrow and the line of best fit for sun exposed remains is indicated by the orange arrow.

6.6.1.1   Sun exposed microclimate

The predicted ADD values found using the Marhoff et al. [2] formula for carcasses in the sun exposed microclimate were compared to the known ADD recorded by the data logger in the sun at the research site.

The linear mixed modelling demonstrated a statistically significant difference between the predicted ADD and the known ADD recorded during Trial 2 using the Marhoff et al. [2] formula on sun exposed remains, with the fixed effect being 0.608 (t (209) = 21.2, $p<0.000$). These results suggest that, on average, the formula underestimated the true ADD during Trial 2. Hence, the known ADD of the sun exposed remains found in this region was 0.608 times greater than the ADD predicted by the Marhoff et al. [2] formula.

Linear regression analysis was performed to determine how well the predicted model was estimating the ADD of the remains in the present study. Regression analysis yielded an r^2 value of 0.804. Using the published guideline that an r^2 value of 0.80 or greater is a reasonably good indicator of accuracy, this would suggest that the Marhoff et al. [2] formula has the potential to reasonably determine the ADD in the Hawkesbury region when remains have decomposed in a sun exposed environment and the temperature at the 'discovery' site is known.

6.6.1.2 Shaded microclimate

The predicted ADD values found using the Marhoff et al. [2] formula for carcasses in the shaded microclimate were compared to the known ADD recorded by the data logger in the shade at the research site.

The linear mixed modelling demonstrated a statistically significant difference between the predicted ADD and the actual ADD recorded during Trial 2 using the Marhoff et al. [2] formula on shaded remains. The fixed effect for shaded remains, expressed as the slope, increased from the intercept (0.608; as recorded for sun exposed remains) by 0.043, resulting in a fixed effect of 0.651 (t (209) = 21.2, p<0.000). The results of the linear mixed model suggest that, on average, the formula overestimated the true ADD during Trial 2. Hence, the predicted ADD using the Marhoff et al. [2] formula was 0.651 times greater than the known ADD value.

Linear regression analysis was performed to determine how well the predicted model was estimating the ADD of the remains in the present study. Regression analysis yielded an r^2 value of 0.798, suggesting the method could be a good predictor of the true ADD of shaded remains in the current study when using temperatures obtained from the 'discovery' site, with further calibration to account for all variables affecting decomposition.
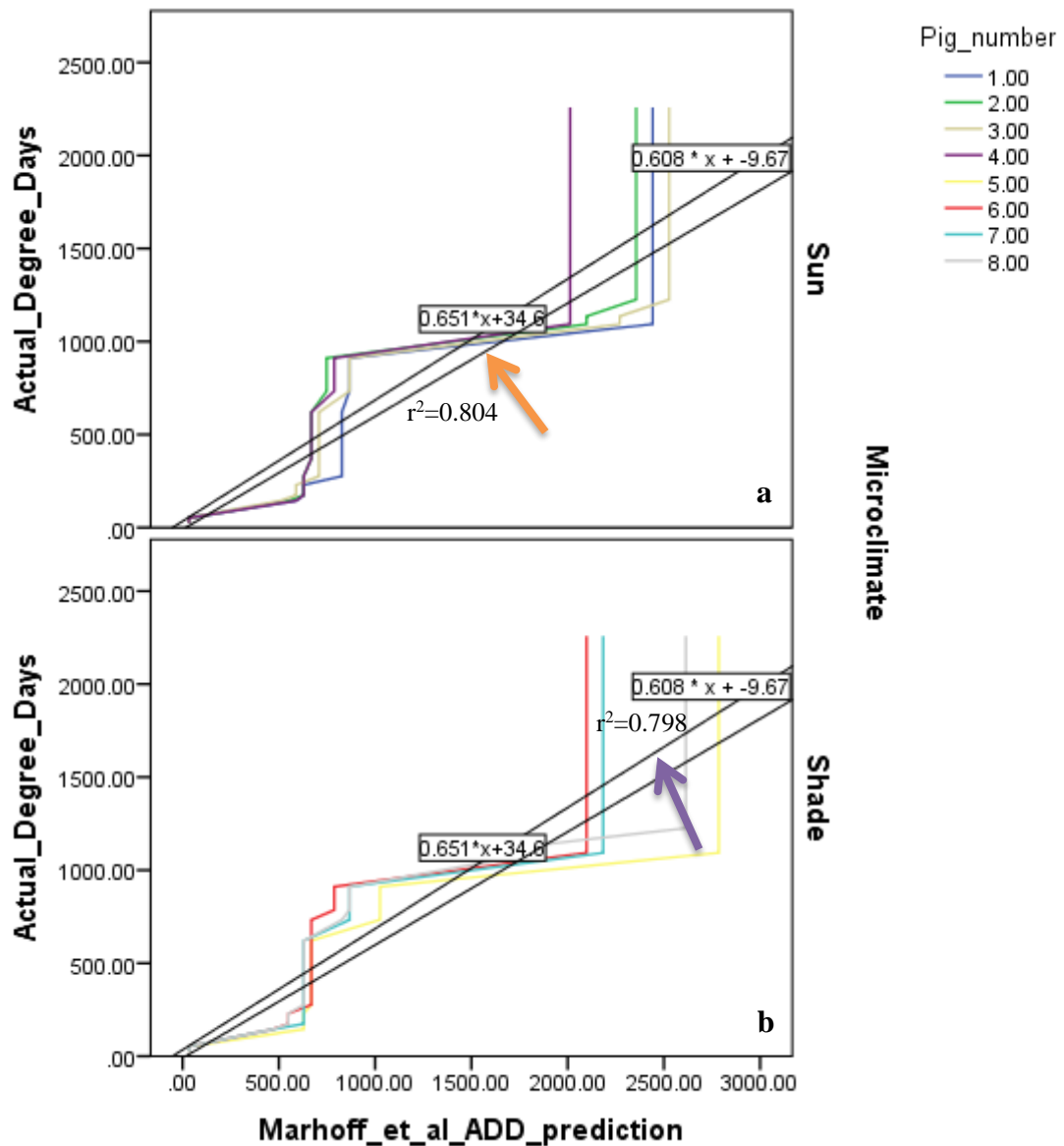
Figure 6-13 The Marhoff et al. [2] ADD prediction vs. the actual ADD of all carcasses (n=8), where 0.608*x+ -9.67 (orange arrow) and 0.651*x +16.04 is the line of best fit for carcasses located in the shade (purple arrow) is the line of best fit for carcasses located in the shade for Trial 2 summer 2014/15. Units in 'days °C'.

6.6.2 Using temperature data collected from the BoM weather station

Figure 6-14 depicts the predicted ADD against the known ADD of remains located in the sun exposed (Figure 6-14a) and shaded (Figure 6-14b) microclimates, using temperature data recorded by the local BoM weather station. Similar to Figure 6-13, it can be observed in Figure 6-14 that both microclimates exhibited similarities in the predicted and true ADD at approximately 700ADD and 1000ADD. At all other time

points the variation between the known and estimated ADD for each carcass was large and the difference between these values increased as time progressed. Due to software limitations, the line of best fit for both the shaded remains and the sun exposed remains appears on both Figure 6-14a and Figure 6-14b. Therefore, the line of best fit for shaded remains is indicated by the purple arrow and the line of best fit for sun exposed remains is indicated by the orange arrow.

6.6.2.1 Sun exposed microclimate

The predicted ADD values found using the Marhoff et al. [2] formula for carcasses in the sun exposed microclimate were compared to the known ADD recorded by the local BoM weather station, using linear mixed modelling.

The linear mixed modelling demonstrated a statistically significant difference between the predicted ADD and the known ADD recorded during Trial 1 using the Marhoff et al. [2] predictive method on the sun exposed remains, with the fixed effect being 0.621 (t (209) =21.1, $p<$ 0.000). The results of the linear mixed model suggest that, on average, the formula overestimated the true ADD of the sun exposed remains during Trial 2. Hence, the predicted ADD using the Marhoff et al. [2] formula was 0.621 times greater than the known ADD value when temperatures were recorded at the local BoM weather station.

Linear regression analysis was performed to determine how well the predicted model was estimating the ADD of the remains in the present study. Regression analysis yielded an r^2 value of 0.802 Using the published guideline that an r^2 value of 0.80 or greater is a reasonably good indicator of accuracy, this would suggest that the Marhoff et al. [2] method has the potential to reasonably determine the ADD in the Hawkesbury region when remains have decomposed in a sun exposed environment and the temperature at the 'discovery' site is known.

6.6.2.2 Shaded microclimate

The predicted ADD values found using the Marhoff et al. [2] formula for carcasses in the shaded microclimate were compared to the known ADD recorded by the BoM local weather station, using linear mixed modelling.

The linear mixed modelling demonstrated a statistically significant difference between the predicted ADD and the known ADD recorded during Trial 2 using the

Marhoff et al. [2] predictive method on the shaded remains. The fixed effect for shaded remains, expressed as the slope, increased from the intercept (0.621; as recorded for sun exposed remains) by 0.041, resulting in a fixed effect of 0.662 (t (208.4) = 0.957, p<0.340). The results of the linear mixed model suggest that, on average, the formula overestimated the true ADD of the shaded remains during Trial 2. Hence, the predicted ADD using the Marhoff et al. [2] formula was 0.662 times greater than the known ADD value, when temperatures were recorded at the local BoM weather station.

Linear regression analysis was performed to determine how well the predicted model was estimating the ADD of the remains in the present study. Regression analysis yielded an r^2 value of 0.797, suggesting the method could be a relatively good predictor of the true ADD of shaded remains in the current study when using temperatures obtained from the local weather station, with further calibration to account for all variables affecting decomposition.
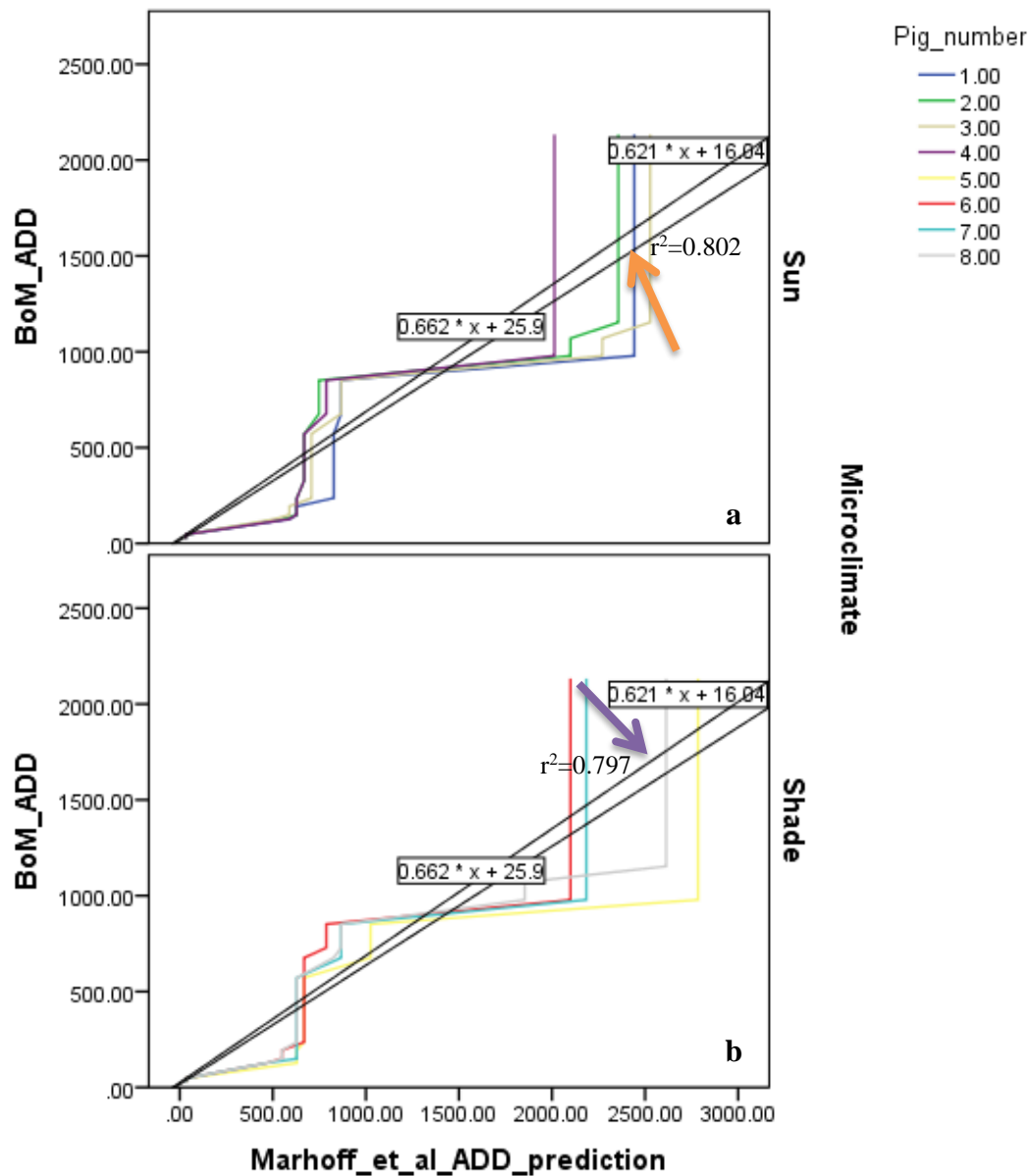
Figure 6-14 Marhoff et al. [2] ADD prediction vs. known ADD of all carcasses (n=8) found using BoM temperature for shade and sun exposed carcasses for Trial 2. Where the line of best fit for PMI sun= 0.662*x+25.9 (orange arrow); PMI shade=0.621*x+25.9 (purple arrow). Units in 'days °C'.

6.7 Validating the Marhoff et al. formula- Trial 4

6.7.1 Using temperature data recorded at the research site

Figure 6-15 depicts the predicted ADD against the known ADD of remains, as recorded by the onsite data loggers, located in the shaded (Figure 6-15a) and sun exposed (Figure 6-15b) microclimates. Two different patterns are observed when the predicted ADD for each microclimate is plotted against the known ADD for Trial 4. Each of the four shaded carcasses decomposed differently to one another and were

subsequently assigned different TBS values. These TBS values alter the estimation of ADD resulting in the variation in estimations between each carcass and the variation between the predicted and known ADD when plotted. The sun exposed carcasses decomposed similarly to one another and, as a result, similar estimations of ADD were found. It can be observed in Figure 6-15 that the predicted and known ADD did not align at any time point for either microclimate.

Due to software limitations, the line of best fit for both the shaded remains and the sun exposed remains appears on both Figure 6-15a and Figure 6-15b. Therefore, the line of best fit for shaded remains is indicated by the purple arrow and the line of best fit for sun exposed remains is indicated by the orange arrow.

6.7.1.1 Shaded microclimate

The predicted ADD values found using the Marhoff et al. [2] formula for carcasses in the shaded microclimate were compared to the known ADD recorded by the data logger in the shade at the research site.

The linear mixed modelling demonstrated a statistically significant difference between the predicted ADD and the known ADD recorded during Trial 4 using the Marhoff et al. [2] formula on shaded remains. The fixed effect for shaded remains, expressed as the slope, decreased from the intercept (2.24; as recorded for sun exposed remains) by 0.150, resulting in a fixed effect of 2.09 (t (212) =-0.144, $p<0.886$). These results suggest that, on average, the formula underestimated the true ADD during Trial 4. Hence, the known ADD of the shaded remains found in this region was 2.09 times greater than the ADD predicted by the Marhoff et al. [2] formula.

Linear regression analysis was performed to determine how well the predicted model was estimating the ADD of the remains in the present study. Regression analysis yielded an r^2 value of 0.578, suggesting the method was a poor predictor of the true ADD of shaded remains in the current study when using temperatures obtained at the 'discovery' site.

6.7.1.2 Sun exposed microclimate

The predicted ADD values found using the Marhoff et al. [2] formula for carcasses in the sun exposed microclimate were compared to the known ADD recorded by the data logger in the sun at the research site.

The linear mixed modelling demonstrated a statistically significant difference between the predicted ADD and the known ADD recorded during Trial 4 using the Marhoff et al. [2] formula on sun exposed remains, with the fixed effect being 2.32 (t (212) = 9.88, p<0.000). These results suggest that, on average, the formula underestimated the true ADD during Trial 4. Hence, the known ADD of the sun exposed remains found in this region was 2.32 times greater than the ADD predicted by the Marhoff et al. [2] formula.

Linear regression analysis was performed to determine how well the predicted model was estimating the ADD of the remains in the present study. Regression analysis yielded an r^2 value of 0.420, suggesting the method was a poor predictor of the true ADD of sun exposed remains in the current study when using temperatures obtained from the local weather station.
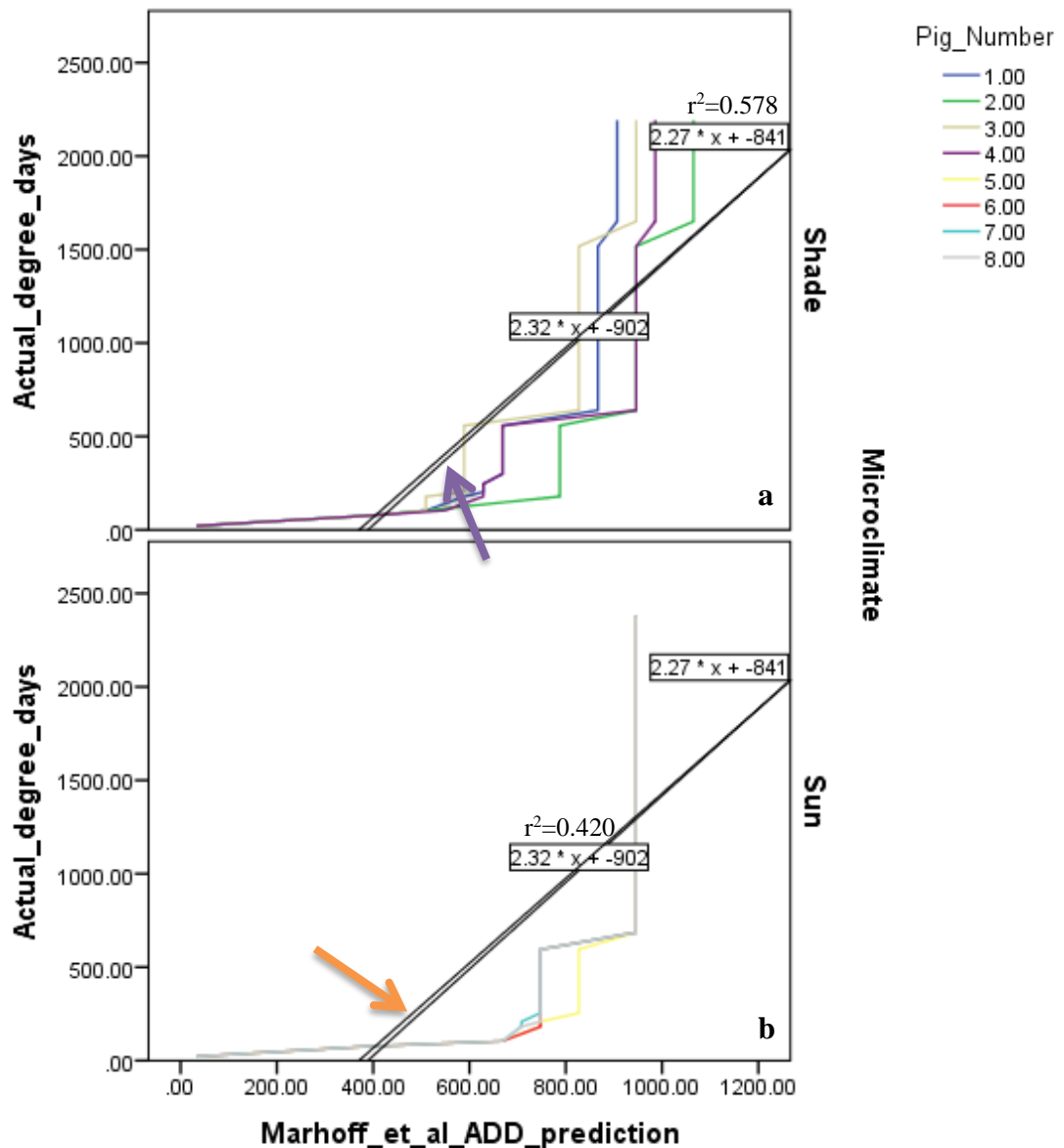
Figure 6-15 Marhoff et al. [2] ADD prediction vs. the known ADD of all carcasses (n=8) found using local weather station temperature for carcasses located in the sun and shade for Trial 4 summer. Where the line of best fit for PMI shade=2.27*x+-841 (purple arrow) and PMI sun= 2.32*x+-902 (orange arrow). Units in 'days °C'.

6.7.2 Using temperature data collected from the BoM weather station

Figure 6-16 depicts the predicted ADD against the known ADD of remains located in the shaded (Figure 6-16a) and sun exposed (Figure 6-16b) microclimates, as recorded by the local BoM weather station. Similar results were found when comparing temperatures obtained at the research site (Figure 6-15) and temperatures obtained from the BoM weather station (Figure 6-16). It can be observed in the graph

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that the predicted and known ADD did not align at any time point for either microclimate.

Due to software limitations, the line of best fit for both the shaded remains and the sun exposed remains appears on both Figure 6-16a and Figure 6-16b. Therefore, the line of best fit for shaded remains is indicated by the purple arrow and the line of best fit for sun exposed remains is indicated by the orange arrow.

6.7.2.1   Shaded microclimate

The predicted ADD values found using the Marhoff et al. [2] formula for carcasses in the shaded microclimate were compared to the known ADD recorded by the local BoM weather station.

The linear mixed modelling demonstrated a statistically significant difference between the predicted ADD and the known ADD recorded by the BoM weather station during Trial 4 using the Marhoff et al. [2] formula on shaded remains. The fixed effect for shaded remains, expressed as the slope, decreased from the intercept (2.24; as recorded for sun exposed remains) by 0.150, resulting in a fixed effect of 2.09 (t (205.9) =9.74, $p<0.000$). These results suggest that, on average, the formula underestimated the true ADD during Trial 4. Hence, the known ADD of the shaded remains found in this region was 2.09 times greater than the ADD predicted by the Marhoff et al. [2] formula.

Linear regression analysis was performed to determine how well the predicted model was estimating the ADD of the remains in the present study. Regression analysis yielded an r^2 value of 0.431, suggesting the method was a poor predictor of the ADD of shaded remains in the current study, when using temperatures obtained from the BoM weather station.

6.7.2.2   Sun exposed microclimate

The predicted ADD values found using the Marhoff et al. [2] formula for carcasses in the sun exposed microclimate were compared to the known ADD recorded by the local BoM weather station.

The linear mixed modelling demonstrated a statistically significant difference between the predicted ADD and the known ADD recorded during Trial 4 using the Marhoff et al. [2] formula on sun exposed remains, with the fixed effect being 2.24 (t

(205.9) =11.3, $p<0.000$). These results suggest that, on average, the formula [2] underestimated the true ADD during Trial 4. Hence, the known ADD of the sun exposed remains was 2.24 times greater than the ADD the Marhoff et al. [2] formula was estimating, when using temperatures obtained from the local BoM weather station.

Linear regression analysis was performed to determine how well the predicted model was estimating the ADD of the remains in the present study. Regression analysis yielded an r^2 value of 0.582, suggesting the method was a poor predictor of the true ADD of sun exposed remains in the current study.
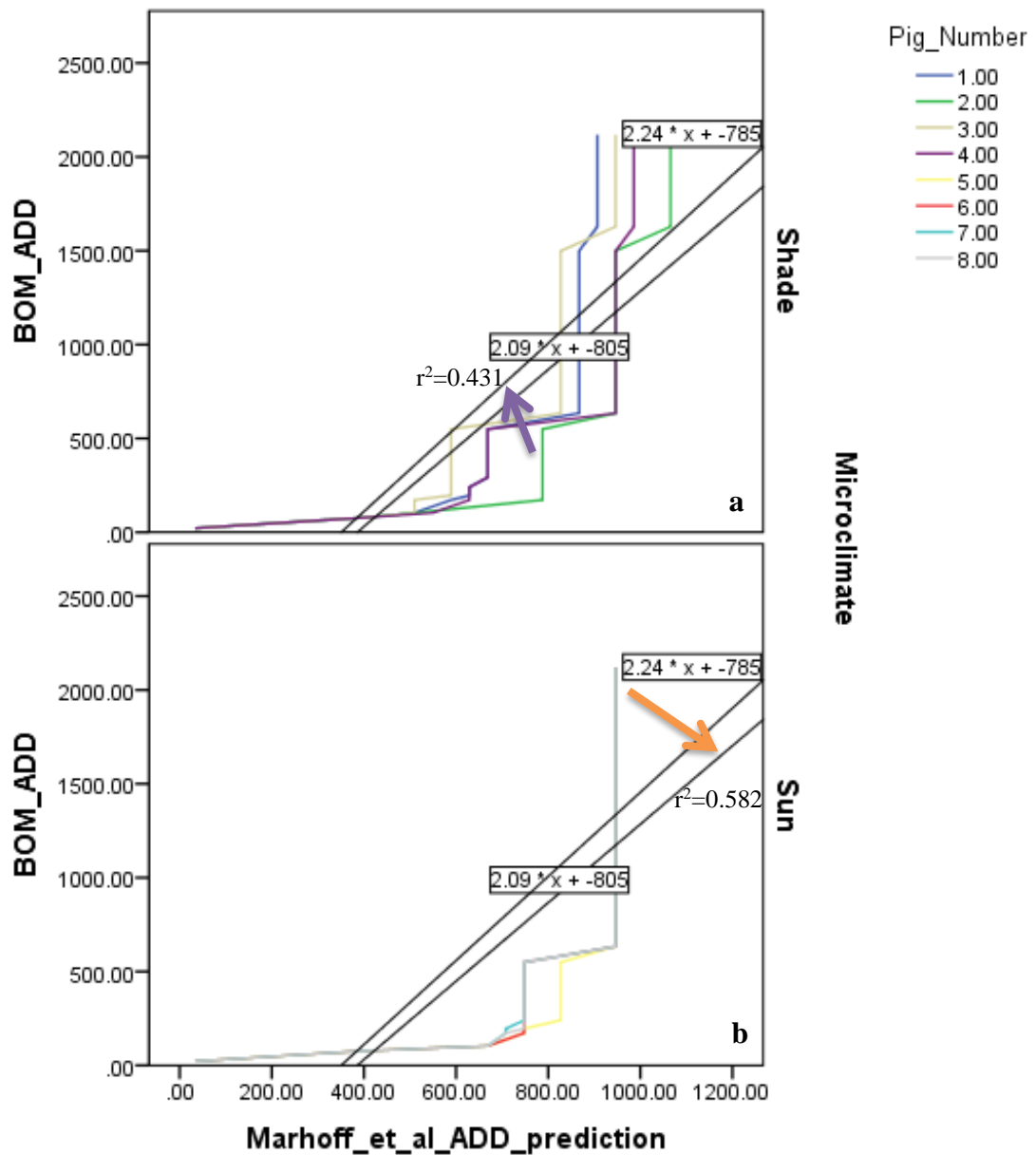
Figure 6-16 Marhoff et al. [2] ADD prediction vs. the actual ADD of all carcasses (n=8) found using data logger temperature for carcasses located in the sun and for carcasses located in the shade for Trial 4 2015/16. Where the line of best fit for PMI shade=2.09*x+-805 (purple arrow); PMI sun= 2.24*x+-785 (orange arrow). Units in 'days °C'.

6.7.3 Summary

The results of the validation of the Marhoff et al. [2] formula indicate this method is not appropriate for accurately determining the PMI of remains in a Western Sydney summer climate. Table 6-3 and Table 6-4 numerically summarise the application of the Marhoff et al. [2] formula at randomly selected 'discovery' dates from Trial 2 and Trial 4, respectively. Results are reported from a single carcass (Pig 1; Trial 2 (shade), Trial 4 (sun)), with full results reported in Appendix J and Appendix P.

Table 6-3 and Table 6-4 show the inaccuracy found when applying the Marhoff et al. [2] formula. This confirms the results observed in Figure 6-13-Figure 6-16, where at no point across the two trials does the predicted and known ADD align. It can be observed in Table 6-3 that at Day 90, the PMI estimate was only 9 days over the true ADD value. However, it can also be seen from the table that the PMI estimate was found to be 99 days from Day 40 to Day 90, highlighting the limitations of this method once remains have desiccated and finally skeletonised.

As Table 6-3 and Table 6-4 demonstrate, when this method is applied using temperatures obtained from the research site and from the local BoM weather station, the difference between the estimations when these data sources is on average 2 days. Neither source of data improved the PMI estimation.

Table 6-3 Summary table comparing the predicted ADD with the known/true ADD for Pig 1 (shade) in Trial 2[*].

PMI (in days)	Predicted ADD[*]: Data Logger	Known ADD: Data Logger	Predicted ADD[*]: BoM	Known ADD: BoM
5	589.10 (23.91 days)	144.80	589.10 (25.13 days)	127.15
10	827.30 (33.57 days)	276.45	827.30 (35.29 days)	236.75
15	827.30 (33.57 days)	392.75	827.30 (35.29 days)	352.65
40	2442.10 (99.11 days)	1093.40	2442.10 (104.18 days)	978.65
90	2442.10 (99.11 days)	2258.25	2442.10 (104.18 days)	2133.27

[*] ADD expressed as days °C. The ADD has been converted to days and appears in the brackets within the table.

Table 6-4 Summary table comparing the predicted ADD with the known/true ADD for Pig 1 (sun) in Trial 4[*].

Day in PMI	Predicted ADD[*]: Data logger	Known ADD: Data Logger	Predicted ADD[*]: BoM	Known ADD: BoM
5	509.70 (21.06 days)	128.40	509.70 (21.65 days)	125.35
10	628.80 (25.98 days)	245.70	628.80 (26.71 days)	240.40
20	668.80 (27.63 days)	471	668.80 (28.41 days)	468.55
40	867 (35.83 days)	917.55	867 (36.83 days)	908.14
90	906.70 (37.46 days)	2191.80	906.70 (38.52 days)	2117.55

[*] ADD expressed as days °C. The ADD has been converted to days and appears in the brackets within the table.

On average, the published method [2] *overestimated* the ADD in Trial 2 by approximately 0.635 times the true ADD value, using both temperatures obtained at the research site and the local weather station. On average, the published method [2] *underestimated* the ADD in Trial 4 by approximately 2.18 times the true ADD value, using both temperatures obtained at the research site and the local weather station.

The results of the validation of the Marhoff et al. [2] method will be compared to the other published methods at the conclusion of this chapter.

6.8   Validating the Vass Universal PMI Formula- Trial 2

6.8.1   Trial 2 (summer) body scored using percentage of decomposition

Figure 6-17 displays the results of the relationship between the percentage of the overall decay on each of the eight carcasses in Trial 2 of the current study, using the Vass [3] formula and PMI, as a means of depicting soft tissue changes semi-quantitatively. It can be seen in Figure 6-17 the decomposition of soft tissue was observed to occur rapidly in the early post-mortem period (Days 5-15) during Trial 2, where it can be seen that the percentage of decay was increasing exponentially as changes were observed on each sampling day during this time period.

At approximately Day 15, decomposition stalled when remains were approximately 60-85% decomposed (as indicated by the orange arrow), this resulted in a plateau from Day 15-40. After Day 40, decomposition became active once again and new morphological changes were observed, however, the decomposition process of remains soon stalled at Day 60 resulting in another plateau when plotted (indicated by the red arrow) and no further changes were observed throughout the length of the trial, concluding at Day 90.
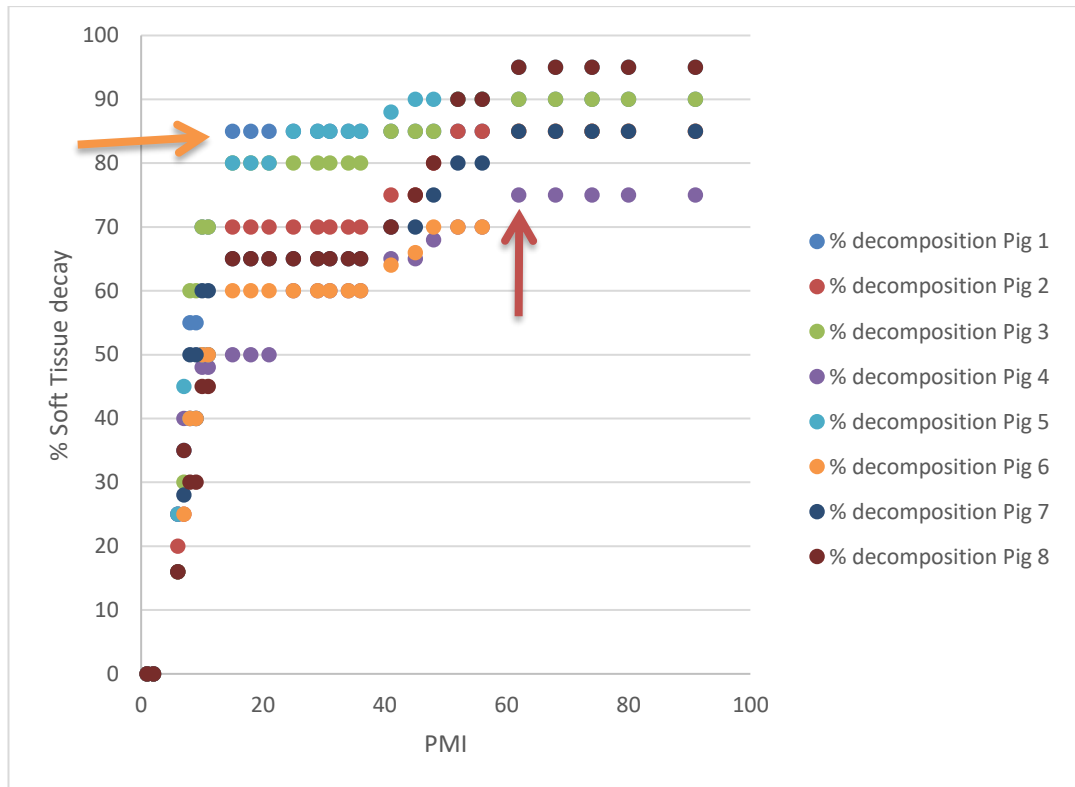
Figure 6-17 Scatterplot demonstrating the progression of soft tissue decay over the PMI for all 8 carcasses in Trial 2 (summer). Orange arrow indicates the beginning of a plateau between Days 15 and 40 and the red arrow indicated a second plateau from Day 60 where no further changes were observed for the remainder of the trial (Day 90)

Figure 6-18 depicts the predicted PMI against the known PMI of remains located in the sun exposed (Figure 6-18a) and shaded (Figure 6-18b) microclimates, where PMI is measured in days. Carcasses in both microclimates exhibited similarities in the variation between the predicted and known PMI. All carcasses were estimated a similar PMI up until approximately Day 40, after which, the PMI estimation varied amongst individual carcasses resulting in some separation between carcasses and the predicted and known PMI.

Due to software limitations, the line of best fit for the shaded remains and the sun exposed remains appears on both Figure 6-18a and Figure 6-18b. Therefore, the line of best fit for shaded remains is indicated by the purple arrow and the line of best fit for sun exposed remains is indicated by the orange arrow.

6.8.2 Sun exposed microclimate

The predicted PMI values found using the Vass [3] formula for carcasses in the sun exposed microclimate were compared to the known PMI of the current study, using linear mixed modelling analysis.

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The linear mixed modelling demonstrated a statistically significant difference between the predicted PMI and the known PMI of the carcasses in Trial 2 when using the Vass [3] predictive formula on sun exposed remains, with the fixed effect being 1.18 (t (205.1) =12.5, $p<0.000$). These results suggest that, on average, the formula underestimated the true PMI during Trial 2. Hence, the known PMI was 1.18 times greater than the predicted PMI using the Vass [3] formula.

Linear regression analysis was performed to determine how well the predicted model was estimating the PMI of the remains in the present study. Regression analysis yielded an r^2 value of 0.439, suggesting the method was a poor predictor of the true PMI of sun exposed remains in the current study.

6.8.3 Shaded microclimate

The estimated PMI found using the Vass [3] formula for carcasses in the shaded microclimate were compared to the known PMI of the remains in Trial 2 of the current study using linear mixed modelling analysis.

The linear mixed modelling demonstrated a statistically significant difference between the estimated PMI and the known PMI of the shaded remains in Trial 2 found using the Vass [3] formula. The fixed effect for shaded remains, expressed as the slope, increased from the intercept (1.18; as recorded for sun exposed remains) by 0.006, resulting in a fixed effect of 1.24 (t (206.7) =-0.407, $p<0.684$). These results suggest that, on average, the formula underestimated the true PMI during Trial 2. Hence, the true PMI was 1.24 times greater than the estimated PMI found using the Vass [3] formula is applied.

Linear regression analysis was performed to determine how well the predicted model was estimating the PMI of the remains in the present study. Regression analysis yielded an r^2 value of 0.627, suggesting the method was a poor predictor of the true PMI of shaded remains in the current study.
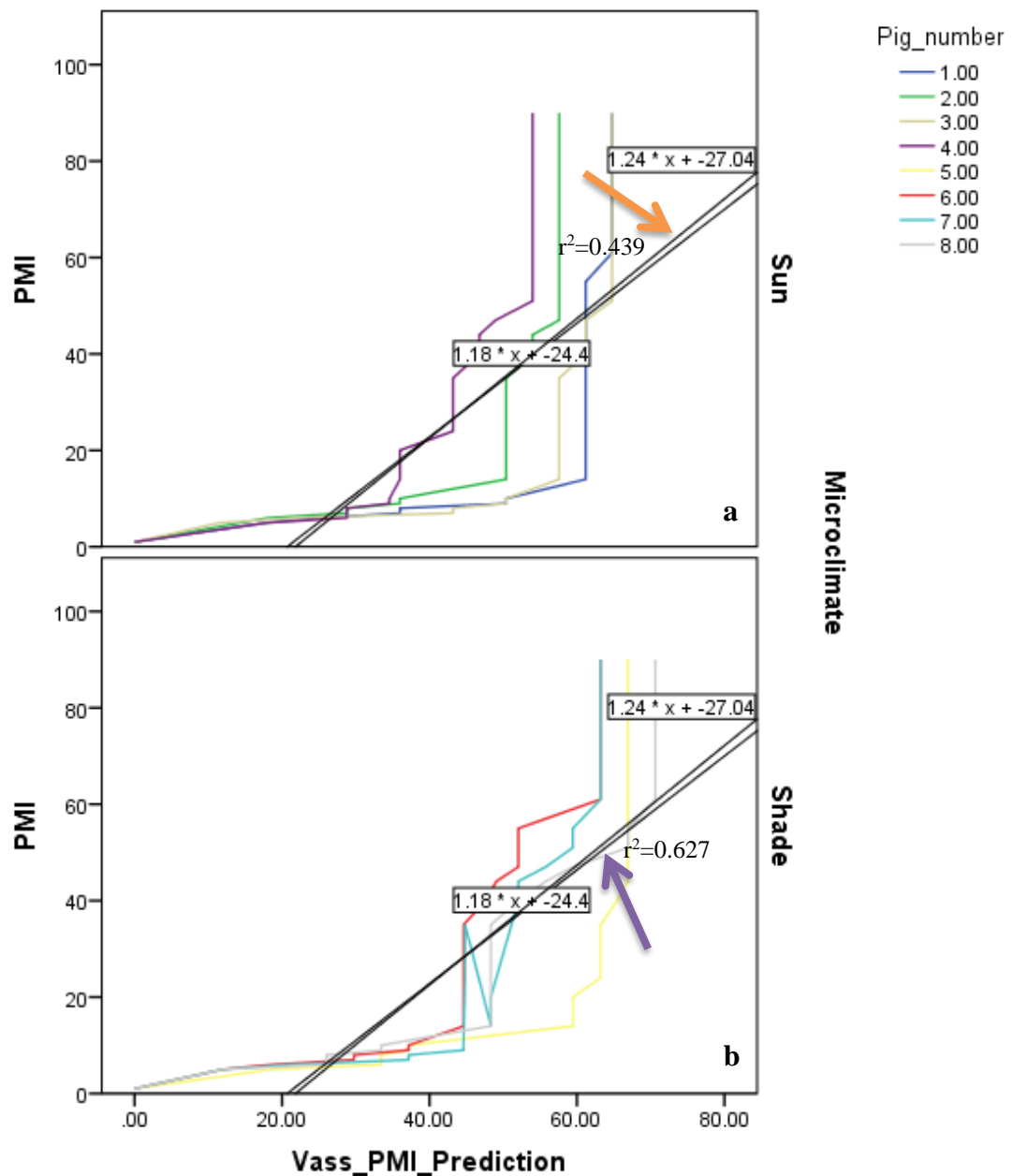
Figure 6-18 The Vass [3] prediction of PMI vs. the actual PMI of all carcasses (n=8) to demonstrate the relationship between the actual and predicted PMI of the carcasses in the sun and the shade. Chart includes the line of best for each microclimate. PMI sun= 1.18*x+-24.4 (orange arrow); PMI shade=1.24*x+-27.04 (purple arrow). Units in 'days'.

6.9 Validating the Vass Universal PMI Formula- Trial 4

6.9.1 Trial 4 (summer) body scored using percentage of decomposition

Figure 6-19 displays the results of the relationship between the percentage of the overall decay on each of the eight carcasses in Trial 4 of the current study, using the Vass [3] formula and PMI, as a means of depicting soft tissue changes semi-quantitatively.

When the percentage of soft tissue decay is plotted against the PMI for Trial 4, a curvilinear pattern is once again generated. Figure 6-19 shows the rapid soft tissue decay early in the PMI between Days 3-8. After this time, the decomposition of remains slowed considerably and entered a period of stasis (Days 8-24), as indicated by the orange arrow.

All eight remains of Trial 4 only actively decayed for a short period of time before mostly skeletonising at Day 27. By Day 27, the percentage of soft tissue decomposition of all eight carcasses was between 75% and 90% decomposed, after which time few morphological changes were observed to occur between Day 27 and 65, appearing as a plateau when charted (as indicated by the blue arrow). Remains actively decomposing at day 65 but soon ceased decaying at Day 70 (as indicated by the purple arrow), a finding that was also observed in Trial 2. The decomposition of remains in Trial 4 did not progress beyond this point and maintained this degree of decay until the end of the trial at Day 90.
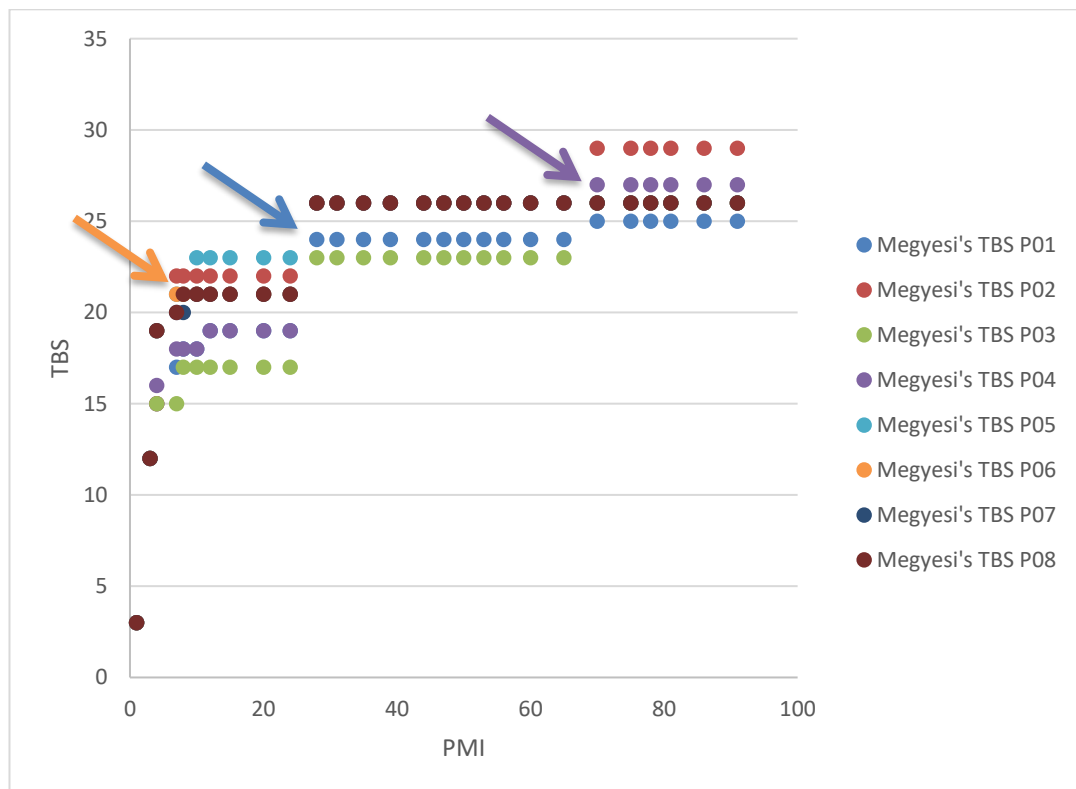
Figure 6-19 Scatterplot of TBS vs. PMI (in days) for all 8 carcasses (Pigs 1-8) to demonstrate the relationship between transpired time and the decomposition of remains in Trial 4. Orange arrow indicates the first plateau between Days 8 and 24. Blue arrow indicates the second plateau ranging from Days 27 and 65. A third plateau is indicated by the purple arrow at Day 70 whereby no further decomposition changes were observed for the remainder of the trial.

Figure 6-20 depicts the predicted PMI against the known PMI of remains located in the shade (Figure 6-20a) and sun exposed (Figure 6-20b) microclimates, where PMI is measured in days. Across most time points, the PMI estimation of the sun exposed carcasses was the same, with only some differences between Days 10-30. Unlike the sun exposed carcasses, the shaded carcasses all received various estimations of the PMI. It can be observed from the graph below that the variation between the predicted and known PMI increased as the trial progressed and did not align at any time point.

Due to software limitations, the line of best fit for the shaded remains and the sun exposed remains appears on both Figure 6-20a and Figure 6-20b. Therefore, the line of best fit for shaded remains is indicated by the purple arrow and the line of best fit for sun exposed remains is indicated by the orange arrow.

6.9.2 Shaded microclimate

The estimated PMI found using the Vass [3] formula for carcasses in the shaded microclimate were compared to the known PMI of the remains in Trial 4 of the current study using linear mixed modelling analysis.

The linear mixed modelling demonstrated a statistically significant difference between the estimated PMI and the known PMI of the shaded remains in Trial 4 found using the Vass [3] formula. The fixed effect for shaded remains, expressed as the slope, increased from the intercept (0.727; as recorded for sun exposed remains) by 0.770, resulting in a fixed effect of 1.50 (t (212) =6.21, p<0.000). These results suggest that, on average, the formula underestimated the known PMI during Trial 4. Hence, the true PMI of remains in the present study was on average 1.50 times greater than the predicted PMI using the Vass [3] formula.

Linear regression analysis was performed to determine how well the predicted model was estimating the PMI of the remains in the present study. Regression analysis yielded an r^2 value of 0.321, suggesting the method was a poor predictor of the true PMI of shaded remains in the current study.

6.9.3 Sun exposed microclimate

The estimated PMI found using the Vass [3] formula for carcasses in the sun exposed microclimate were compared to the known PMI of the remains in Trial 4 of the current study using linear mixed modelling analysis.

The linear mixed modelling demonstrated a statistically significant difference between the estimated PMI and the known PMI of the sun exposed remains in Trial 4 found when using the Vass [3] formula, with the fixed effect for sun exposed remains being 0.727 (t (212) = 7.01, $p<0.000$). These results suggest that, on average, the formula overestimated the true PMI during Trial 4. Hence, the Vass [3] formula predicted the PMI to be 0.727 times greater than the known PMI value.

Linear regression analysis was performed to determine how well the predicted model was estimating the PMI of the remains in the present study. Regression analysis yielded an r^2 value of 0.250, suggesting the method was a poor predictor of the true PMI of sun exposed remains in the current study.
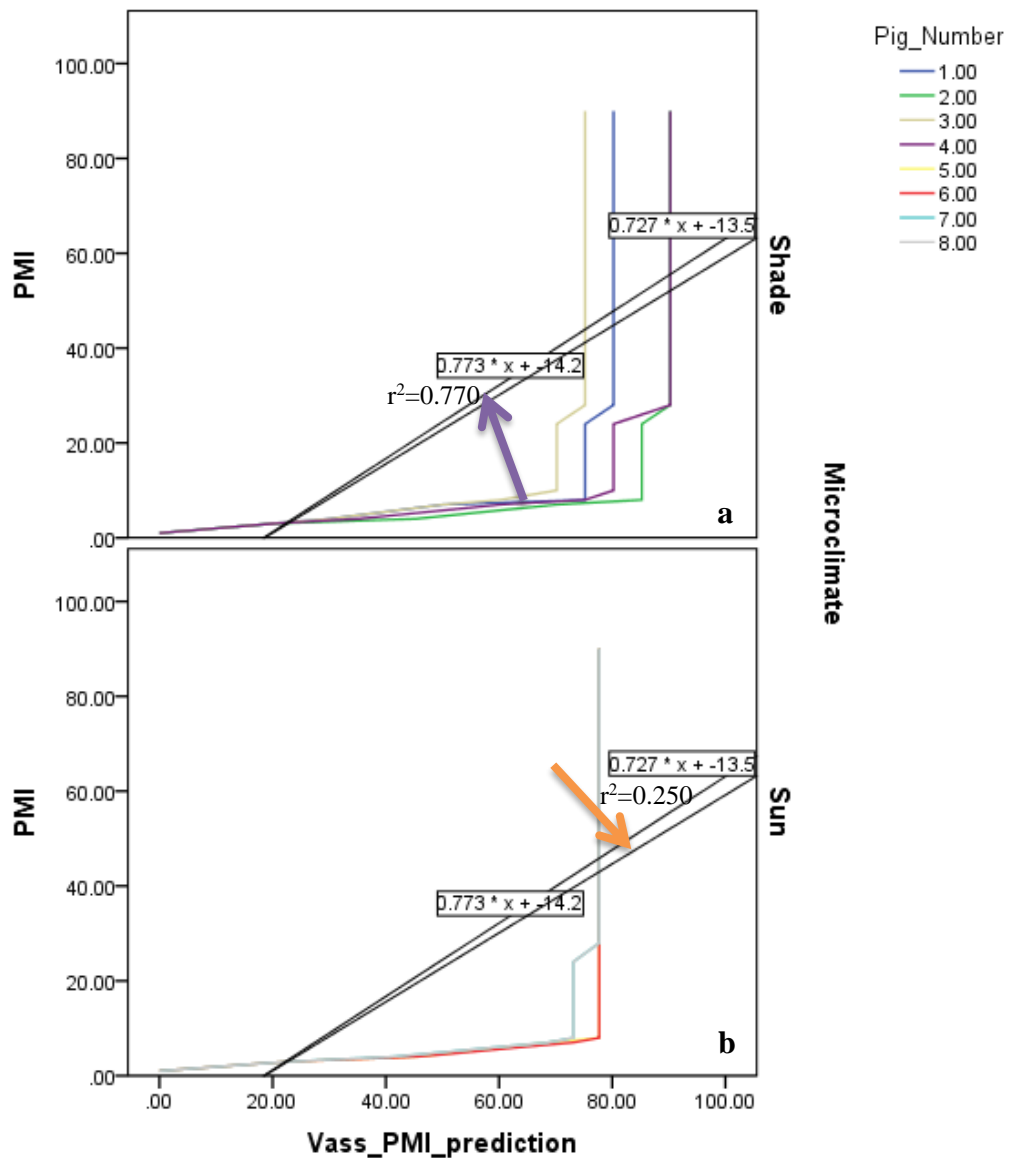
Figure 6-20 The Vass [3] PMI prediction vs. the known PMI of all carcasses (n=8) to demonstrate the relationship between the actual and predicted PMI of the carcasses in the sun and the shade. Chart includes the line of best for PMI shade=0.773*x+-14.2 (purple arrow); PMI sun= 0.727*x+-13.5 (orange arrow). Units in 'days'.

6.9.4 Summary

The results of the validation of the Vass [3] formula for determining the PMI indicates that it cannot accurately determine the PMI of remains deposited in the summer climate of Western Sydney. While accuracy was observed when this method was applied during the winter trials, this method was found to largely be inaccurate in its PMI determinations during the summer trials. Vass [3] suggests this method cannot be applied to skeletonised remains, as such, this factor may have contributed

to the inaccuracies found in the present study as all carcasses across the two summer trials skeletonised early beginning at approximately Day 27.

Table 6-5 numerically summarises the application of Vass' method at randomly selected 'discovery' dates from Trial 2 and Trial 4. Results are reported from a single carcass (Pig 1; Trial 2 (shade), Trial 4 (sun)), with all results reported in Appendix K and Appendix Q.

Table 6-5 Summary table comparing the predicted PMI with the known PMI for Pig 1 (shade) in Trial 2 and Pig 1 (sun) in Trial 4.

True PMI (days)	Predicted PMI (days): Trial 2	Predicted PMI (days): Trial 4
5	23.43	50.14
10	93.37	75.22
15	63.26	75.22
40	60.36	80.23
90	53.07	80.23

On average, the published method underestimated the PMI in the two summer trials by approximately 1.16 times the true PMI value. Initially in the decomposition process, the formula underestimated the PMI, however, as decomposition continued and eventually entered a period of stasis, the formula overestimated the PMI once remains skeletonised.

6.10 Discussion

Of the three methods evaluated for use in temperate Australia, specifically when temperatures are warmer as in the summer months, none could accurately calculate the time since death of the remains used in the present study, regardless of whether data from the research site was used in the analyses or if it was obtained from the BoM. This is likely due to the rapid onset of decomposition where the majority of soft tissue decomposition occurred within a very short period of time of approximately 9-14 days when compared to a steady increase in decomposition as observed during cooler conditions such as the winter trials in this study. While decomposition continued after this time point it was slow and stalled often as

remains were skeletonising. Given the high degree of decomposition early in the post-mortem period, it is possible these methods could not account for such a rapid progression of changes resulting in the overestimation of the PMI by all three methods.

Two of the three methods evaluated in the present study were developed internationally. It has been postulated by many researchers that decomposition is a geographically dependent process and results achieved in these studies may not be applicable or reproduced in all other regions worldwide [12, 16-18, 75]. Therefore, perhaps the variables affecting decomposition in the summer months in temperate Australia are not accounted for by the methods tested, resulting in the erroneous PMI predictions observed above.

The Megyesi et al. [1] method resulted in many inaccuracies and a large range of predicted ADD values that either grossly underestimated or overestimated the ADD, depending on the carcass. For example, when the true ADD was approximately 2220 Days ℃ (Day 90), it predicted values ranging from 737.9 to 18197 Days ℃ which is equivalent to between 30-752 days. A PMI range between 1 month and 2 years is not an accurate determination and cannot assist law enforcement in narrowing a time period to begin investigating.

In Trial 4, however, the Megyesi et al. [1] method performed well, estimating the PMI to with days of the known PMI value at some time points. Trial 4 was the only trial this method performed well in from each of the four trials. The large discrepancy in its performance across trials and seasons suggests there are further factors affecting the accuracy of the method. The method was originally created using decomposed human remains, however the present investigation attempted to validate the method on a porcine model. The accuracy of this method may have been impacted by the use of pig carcasses and as it was not created to be used on a porcine model this may have been a factor in its limitations. This finding has been found previously by Keough et al. [119] where they suggested perhaps a modified scoring protocol may be required if the Megyesi et al. [1] method is to be tested on pig remains.

While difficulties were found in the winter trials, assigning a TBS value to a set of remains was increasingly difficult in the summer trials. Many of the changes outlined in the scoring protocol were missed or did not occur because decomposition was so

rapid. This made it difficult to assign one point value to a carcass, especially when the changes observed were not outlined, subsequently, leading to inaccurate PMI estimates.

The Marhoff et al. [2] formula performed best in Trial 2. With regards to its performance in a shaded or sun exposed microclimate, it more accurately estimated the ADD of remains located in the shade. On average in Trial 4, the method overestimated the ADD by 0.635 times. This variation between the predicted and actual ADD narrowed and became closer to a one-to-one relationship when remains entered the advanced decomposition stage regardless of the microclimate; a similar result was also found in the winter trials of the present study. This suggests the method could better predict the PMI when remains were classified in the advanced decomposition stage.

The Marhoff et al. [2] method also makes use of the original scoring protocol designed by Megyesi et al. [1] given the different changes observed in the present investigation, perhaps the method could perform better if a new scoring protocol specific to the decomposition changes observed in Australia was created. As this may eliminate some of the subjectivity associated with trying to fit a stage of decay when the changes are not actually observed.

The Vass [3] method did not perform as well in the summer trials as it did during the winter trials. Decomposition occurred rapidly as discussed previously, causing the method to overestimate the PMI from the early decomposition stage but underestimate the PMI once remains had skeletonised. Because this formula assumes that the greater the percentage of decomposition observed is related to a greater number of days passing in the PMI, it fails to identify the rapid onset of decay observed when remains are exposed to warmer conditions and, as such, predicts the PMI to be greater than what it is, due to the greater levels of decomposition observed earlier.

It is possible this formula failed to precisely estimate the true PMI of these remains as the constants (moisture and a standard ADD remains are cease decomposing) which make up the variables in this formula may not reflect the values of these variables in the Greater Western Sydney region. Cockle and Belle [50] also suggest these values may need to be refined for each geographic location to successfully apply the Vass [3] formula.

Vass [3] proposes this formula should provide a rough estimation of the PMI to allow investigators to begin their investigation. This formula largely underestimated and overestimated the PMI in the summer trials but could be successfully applied in the winters. Such varying results make it difficult to apply this method

Due to the inaccuracy found when applying each of the currently published methods, it has been concluded that a new method for narrowing PMI estimations of remains deposited in the summer will be created based on the data obtained in the present study. This method will be made specific for use of PMI calculations in the Greater Western Sydney region in an attempt to narrow PMI estimates of remains.

Chapter Seven

7 Development and validation of a new predictive model for estimating PMI

7.1 Introduction

As it was observed in Chapters 5 and 6 that the three current methods for PMI estimations [1-3] could not accurately estimating the time since death of remains in the current study, it was determined a new equation needed to be developed that more accurately estimated the PMI for remains deposited in the Greater Western Sydney area. As mentioned in the previous chapters, the methods evaluated in the present study were tested using weather data obtained at the research site and from the local BoM weather station. This was to determine if the data recorded for the same variable (e.g. temperature) was consistent between the two sites and if one recording device could produce better accuracy when its data was inputted into the formula to generate a prediction. When these methods were applied using the data recorded at the site of the remains and from the BoM weather station, it was found that the methods did not accurately determine the ADD any better using either of these means (Appendix F-Appendix Q). As the current methods for PMI determinations did not accurately determine the PMI, this provided purpose and validity to develop a new method, titled the Marhoff-Beard method, for PMI estimations that is made specific for remains recovered in the Greater Western Sydney region.

The following chapter describes:

(i)   the process of creating the new Marhoff-Beard method for PMI calculations (Section 7.2),

(ii)   applying the criteria of the Marhoff-Beard method to decomposed remains (Section 7.2.1 and Section 7.2.2),

(iii)   the accuracy and replicability of the Marhoff-Beard method when validated on a porcine model from alternate winter and summer subsets (Section 7.3),

<ol type="i" start="4">
the accuracy and replicability of the Marhoff-Beard method when validated on human remains from photographic images (retrospectively) (Section 7.4),
the accuracy and replicability of the Marhoff-Beard method when validated on a human body (longitudinally) (Section 7.5).

7.2 Creating a new equation for PMI estimations

When analysing the decomposition process observed in the two seasons studied over two years, it was found that when decomposition was quantified as either TBS or represented as a percentage of decay, the calculated ADD and PMI and the associated accuracies was very different between the two seasons studied (summer and winter). During the summer trials, higher TBS and percentage of decay values were achieved rapidly in a shorter time period whereas in the winter trials, these values increased in smaller increments over the entire course of the study (90 days). Furthermore, it was found that all carcasses that were killed and deposited during the two winter trials, all mummified with no exceptions and all sixteen remains killed and deposited in the two summer trials all skeletonised.

The speed and pattern of decomposition was so different between seasons, as was the climatic data (e.g. temperature, humidity and rainfall), that the creation of a single formula could not account for the vast amount of variation if the data was pooled from those two seasons. When the climatic data and its relationship to PMI is examined within the individual seasons and between the two microclimates, a much closer statistical relationship was found. These differences and key findings led to the conclusion that two seasonally specific models would be developed. That is; microclimate specific (sun/shade) equations would be developed for the calculation of PMI for remains deposited in winter and microclimate specific (sun/shade) equations would be created for remains deposited in the summer using the key characteristics described above. That is when the remains are either skeletonised or mummified, to determine which equation to use and when.

Throughout the course of the study, the data of a multitude of climatic variables that have been suggested in the literature to significantly impact the decomposition process were collected [9, 16, 41, 42, 65, 75, 76]. As it was found that neither the weather data obtained at the research site or from the local weather station improved

the accuracy of the previously published methods, it was concluded that data from the local BoM weather station would be used when creating and applying the new method. This is because weather data from the local weather station is publicly accessible and likely the only source of data available to forensic investigators. Using the weather data from the BoM weather station, it was first determined which variables were statistically significant and, therefore, most influenced the decomposition process in order to include them in the new equation. Through regression analysis it was determined that average daily humidity levels, rainfall, wind speed and microclimate most affected the degree and process of soft tissue decomposition (Table 7-1 and Table 7-2). Unlike the previously published methods evaluated in the current study, the Marhoff-Beard method does not make use of the temperature variable as it was found to not be statistically significant in this investigation.

Table 7-1 and Table 7-2 are summary tables of the regression analyses (extracted from SPSS) displaying the significance of each of the climatic variables on the decomposition process in the present study for the winter and summer trials. The table also presents the coefficients used in the Marhoff-Beard winter and summer formulas ('Unstandardized Coefficients B'), respectively. It can be observed in the tables below (highlighted in yellow) that temperature was not found to be statistically significant for winter or summer.

Table 7-1 Summary table of the significance of the climatic variables on decomposition and the coefficients used in the new winter formula for PMI determinations

Coefficients[a,b]

Model	Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.	95.0% Confidence Interval for B Lower Bound	95.0% Confidence Interval for B Upper Bound
1 (Constant)	2.70903	3.628		12.084	.000	36.711	50.974
Log10_DECOMP	0.721	.025	.878	44.500	.000	1.077	1.176
Log10_rainfall	.0941	.098	.019	.978	.028	-.097	.289
Log10_windspeed	-.4850	.039	-.054	-2.622	.009	-.179	-.026
log10_humidity	-.7144	.035	-.174	-8.868	.000	-.378	-.241
Microclimate	-0.0570	.992	-.115	-6.222	.000	-8.124	-4.224
log10_temperature	.132	.220	.012	.602	.548	-.299	.564

a. Dependent Variable: Log10_PMI

b. Selecting only cases for which Season = Winter

Table 7-2 Summary table of the significance of the climatic variables on decomposition and the coefficients used in the new summer formula for PMI determinations

Coefficients[a,b]

Model	Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.	95.0% Confidence Interval for B Lower Bound	95.0% Confidence Interval for B Upper Bound
1 (Constant)	2.941	6.579		-1.083	.280	-20.055	5.810
Log10_DECOMP	.8227	.046	.666	17.990	.000	.733	.913
Log10_rainfall	-.1289	.076	-.066	-1.689	.002	-.279	.021
Log10_windspeed	-.4966	.098	-.187	-5.052	.000	-.690	-.303
log10_humidity	.0475	.048	.038	.999	.000	-.046	.141
log10_temperature	1.693	.463	.155	3.659	.318	.783	2.602
Microclimate	-7.123	1.976	.054	1.488	.037	-.944	6.825

a. Dependent Variable: Log10_PMI

b. Selecting only cases for which Season = Summer

To create a new regression formula, the variables to be included in the new algorithm, percentage of decomposition, microclimate, humidity, rainfall, and wind speed, were first log transformed. This was to produce a more linear curve as a means to facilitate the use of linear regression analysis for the development of a formula and provide a better understanding of the relationship between the climatic variables, degree of decay and PMI. Using linear regression analysis in SPSS, the variables mentioned above were regressed and the values produced became the co-

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efficient of each of these variables for the new seasonal equations. Following the rules of regression, the positive integers became the numerators and the negative integers became the denominators of the equation [pers. comm. Russell Thomson, Western Sydney University]. In addition, microclimate was also regressed to assist in creating seasonal and microclimate specific equations. To create seasonally specific equations, for summer and winter deposited remains using linear regression in SPSS, 1 was coded for winter and 2 was coded for summer.

Using the determined regressions, the newly developed predictive equations for winter are as follows, where the value produced is the PMI in days[1]:

Equation 4 $PMI_{shade} = ((10)^{2.7-0.057}) * ((\% \text{ decomposition } +1)^{0.72}) * ((\text{rainfall}+0.1)^{0.094}) / ((\text{wind speed})^{0.48}) * ((\text{humidity})^{0.71})) -1$

Equation 5 $PMI_{sun} = ((10)^{2.7}) * ((\% \text{ decomposition } +1)^{0.72}) * ((\text{rainfall}+0.1)^{0.094}) / ((\text{wind speed})^{0.48}) * ((\text{humidity})^{0.71})) -1$

The newly determined equations for summer are:

Equation 6 $PMI_{shade} = ((10)^{2.9-4.2}) * ((\%\text{decomposition}+1)^{0.82}) * ((\text{humidity})^{0.047}) / ((\text{rainfall}+0.1)^{0.13}) * (\text{wind speed})^{0.49})) -1$

Equation 7 $PMI_{sun} = ((10)^{2.9}) * ((\%\text{decomposition}+1)^{0.82}) * ((\text{humidity})^{0.047}) / ((\text{rainfall}+0.1)^{0.13}) * (\text{wind speed})^{0.49})) -1$

By raising 10 to the value expressed in the parentheses, the equation can be expressed as PMI= $10^{\wedge}$ (constant minus the constant for microclimate) to be made specific for estimating the PMI of remains in a *shaded* microclimate. Due to the rules of regression analysis, this process was not necessary for the development of an equation specific to a *sun exposed* microclimate as $10^{\wedge}$ (constant) currently represents the sun exposed microclimate.

The addition of '1' to the percentage of decomposition and the addition of '0.1' to the level of rainfall will account for when soft tissue decomposition is not observed

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[1] While the coefficients are more accurate than the integers in these equations (i.e. use of decimal places compared to whole numbers), the significant figures are consistent with previously published methods. The final reporting value is in whole numbers which complies with the rate limiting step.

to have commenced and when rainfall has not occurred. These variables would otherwise be represented as '0' and, therefore, the equation could not accurately estimate PMI without a value present for either of these variables. The '1' is then subtracted from the final estimation of PMI to account for its addition to the percentage of decomposition.

Algorithms based on *seasonal* data from the present study are described below in Section 7.3. To obtain more accurate calculations of PMI and assist forensic anthropologists when applying the new algorithm, an explanation of the criteria for estimating the percentage of soft tissue decomposition and for obtaining climatic data is outlined below.

7.2.1 Guidelines for estimating the degree of soft tissue decomposition for the Marhoff-Beard method

The anthropologist first takes into account the entire body and makes a visual assessment of how much of the external surface of the body has undergone decomposition. The degree of decay is then allocated a total score by the investigation. For example, if the remains are approximately 15% decomposed, this will be expressed as the integer '15' within the equation.

To make an estimation of the degree of soft tissue decay, the body is divided into five regions as shown in Figure 7-1 when applied to pig carcasses for the development of this method. The five regions the body is divided into are as follows:

- ❖ head and neck
- ❖ arms
- ❖ thorax,
- ❖ abdomen
- ❖ legs, pelvic and gluteal regions.

To allocate a score to a body region as outlined in Figure 7-1, each individual region was allocated a surface area percentage based on how much that body region contributes to the body as a whole. These values are based on the 'Rule of Nine's' which is more commonly used in a clinical setting to measure the surface area of burns to the dermis of a human body [120]. The values assigned to each body region are presented in Figure 7-1. If a body is lying in a position where only one surface of the body is observable (e.g. in the supine position where the back of the body cannot

be seen), it is to be assumed the underbody has decomposed similarly to the exposed surface until the underbody can be observed. These values are summated to give a final percentage of decay to be applied to the equation.

While some scoring protocols combine the arms and the legs as one region (i.e. the 'limbs' region in the Megyesi et al. [1] protocol), it was found in the current study that these regions decomposed independently of one another (see Chapter 3.2.3 and 4.6.2) and should, therefore, be assessed independently for the degree of decay present and for the ease of making the assessment.

To estimate the degree of soft tissue decomposition, the forensic anthropologist should observe each of the five regions mentioned above and make an assessment on all of the structural or gross morphological changes that have occurred to the body during the decomposition process. Changes to assess include the amount of soft tissue that has decomposed, the loss of biomass and structural integrity of the body, skin slippage and degree of bone exposure. It should be noted that skin discolouration should also be included in the assessment criteria as although it is not a structural change that occurs to the body, it is an indication that the body is currently undergoing the decomposition process internally (autolysis).

It should also be determined (if possible depending on the state of decay) if any peri-mortem trauma, ante-mortem trauma or post-mortem trauma has occurred and is present on the body. If any of the above is present, this should not be included in the assessment of decay but serve as an identification tool to determine if decomposition is greater in these areas. This is for consideration when estimating the degree of decay, as the literature suggests trauma sites will decay faster than areas and bodies without damage to the soft tissues [9, 25, 35, 47].
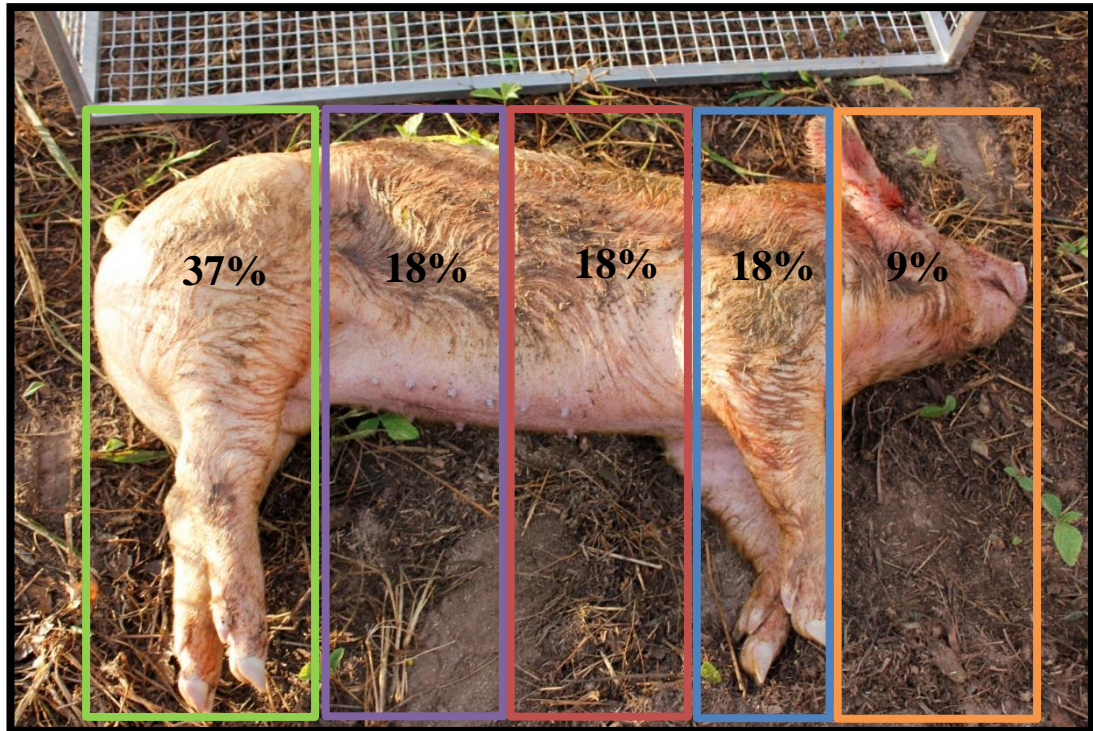
Figure 7-1 The division of a pig carcass into five body regions for estimating the percentage of decomposition that has occurred. ORANGE is the head and neck region, BLUE is the forelimbs, RED is the thoracic region, PURPLE is the abdomen and GREEN is the hind limbs, pelvic and gluteal region. Method based on the 'Rule of Nine's' burns assessment method (Livingston and Lee, 2000).

7.2.2 Obtaining climatic data

The average daily humidity levels, rainfall levels and average wind speed data on the day the body was discovered must be obtained from the local weather station that is closest to the site of the remains, to be included in the formula.

To input these variables in the Marhoff-Beard formula they must be expressed how they were recorded. For example, if the average humidity levels are 95%, rainfall levels were 1.6mm and the average wind speed was 34km/h on the day of discovery, in the formula these values will be expressed as the following integers: 95, 1.6 and 34.

Using data obtained from the local weather station, means it is available to the public and readily available for immediate application within the Marhoff-Beard equation. Hence, an estimate of PMI could be made in the field by the forensic anthropologist much more quickly when compared to the impracticality of using data loggers to record on site weather data on the day the remains are discovered.

7.2.3 Applying the new method to a porcine model

Once an estimation of the degree of soft tissue decay has been made and the climatic data from the day of discovery has been obtained from the local weather station (BoM), these values can be entered into the appropriate Marhoff-Beard formula to generate an estimate of PMI. If the remains are observed to be in the early decomposition stage, the seasonally specific equation can be applied depending on the current season when remains are discovered. If the remains appear in a more advanced state of decay, one should note whether the remains are mostly mummified or mostly skeletonised. If the remains are mummified, the winter equation should be used and if the remains are skeletonised, the summer equation can be used. This is because in the present study we observed all winter remains to mummify and all summer remains to skeletonise without exception in the Western Sydney region. If the microclimate (sun or shade) is known or can be extrapolated from the photographs of the body, the microclimate specific equation can be applied.

Below, we present a hypothetical scenario where the Marhoff-Beard method was applied to estimate the PMI of a set of pig remains decomposing as part of a different study on the same site (i.e. these remains were not used in the development of the Marhoff-Beard equations).

A set of remains has been discovered in the bush under the canopy of trees during winter in 2016. The body is estimated to be 15% decomposed due to the bone exposure of the jaw, skin slippage of the fore limbs, carcass is in the post-bloat stage with a dark green-black discolouration on the neck, thorax and hind limbs and the abdomen has split at the soil level (Figure 7-2). On the day of discovery, the humidity levels were at 58%, 10.8mm of rain fell and wind speeds were approximately 48km/h.
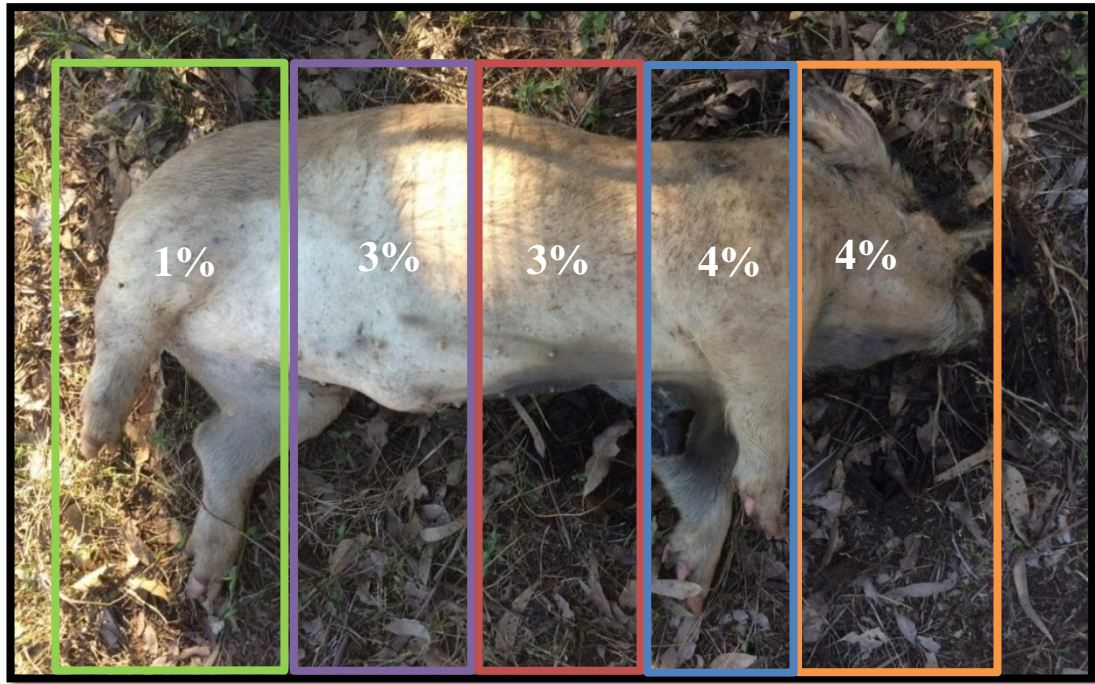
Figure 7-2 A carcass found deposited during winter 2016, estimated to be approximately 15% decomposed. The estimated degree of decay for each body region is demonstrated in the image.

To estimate the PMI of this set of remains, the formula estimates the PMI as follows:

$PMI_{shade} = ((10)^{2.7-0.057}*((\%decomposition+1)^{0.72}) *((rainfall+0.1)^{0.094})) / ((wind\ speed)^{0.48})*((humidity)^{0.71})) -1$

$PMI_{shade} = (439.54*((15+1)^{0.72}) *((10.8+0.1)^{0.094})) / ((58)^{0.48})*((48)^{0.71})) -1$

$PMI_{shade} = (439.54*(7.36) *(1.25)) / ((6.96)*(15.62)) -1$

$PMI_{shade} = (4043.76/108.71) -1$

$PMI_{shade} = 37 -1$

$PMI_{shade} = 36$ days

Therefore, in the example above when the body that was discovered in the aforementioned conditions, the formula estimated the PMI to be approximately 36 days. The known PMI of the carcass in this example was 35 days. This carcass was deposited on June 20 2016 and the 'date of discovery' (when the image was taken) was July 25 2016.

To clarify, as this validation study was a retrospective study performed using images of decomposed remains, from this point the 'date of discovery' refers to when the

various images were taken. These 'dates of discovery' were not known to the author until after the percentage of decomposition for each set of remains was scored, so as not to introduce bias.

7.3   Validation of the new predictive equation on a porcine model

To determine if the Marhoff-Beard method could accurately estimate the PMI of remains and to evaluate its replicability, fourteen photographic images of decomposed pig carcasses, with a known PMI, from an alternate winter subset (nine carcasses) and an alternate summer subset (five carcasses) were obtained (i.e. carcasses were not from the present study).

The carcasses deposited in the alternate winter trial, were placed at the same research site used in the current study at Western Sydney University, Hawkesbury campus on June 20 2016.

The carcasses deposited during the summer, were placed at a research site in Yarramundi, NSW, 14.9km from the current study location (GPS co-ordinates 33°38S, 150°39E). The summer remains were from two different trials, where two carcasses were deposited on January 15 2013 and the other three carcasses were deposited on January 14 2014, to form part of two separate summer studies.

Carcasses at both research sites were placed in a low lying grassed area to naturally decompose on an environmental surface that is common throughout the Western Sydney region. All carcasses from the alternate winter and summer subsets, were obtained from the same abattoir as those carcasses used in the current study and were all killed by a captive head bolt to the brain. All pigs were approximately 60kg.

The following section presents the results of the validation study of the Marhoff-Beard formulas for PMI determinations when applied to a porcine model. Due to the samples being randomly selected from other research projects and small, uneven sample sizes in each validation group, all data will be presented as tables for the results of the validation study unlike in the previous chapters.

7.3.1   Validating the winter equation on a porcine model

Before a calculation of PMI could be made, the climatic data needed to be obtained and a visual assessment of the carcass pictured needed to be made following the criteria outlined in Section 7.2.1. Table 7-3 represents the various climatic data

collected on the 'date of discovery' of the remains, the observed degree of decay of each carcass and the known/true PMI of each set of remains at the time of the 'date of discovery'. All nine carcasses that were used to validate the winter Marhoff-Beard equation were deposited on the soil surface on June 20 2016.

Table 7-3 Summary table of climatic values, total percentage of decay and known PMI of validation group of pig carcasses from winter 2016.

Pig	% decay	Rainfall (mm)	Wind speed (km/h)	Humidity (%)	Date of Discovery	True PMI (days)
1	5	0	13	60	4/7/16	15
2	5	3.6	24	98	8/7/16	18
3	15	10.8	57	48	25/7/16	35
4	15	6.8	26	73	5/8/16	45
5	15	0	35	46	18/7/16	28
6	70	0	35	22	6/10/16	122
7	30	0	48	37	26/9/16	112
8	30	0	28	50	12/9/16	97
9	30	0	22	22	6/10/16	122

Using the data listed above in Table 7-3, these values were placed in the appropriate Marhoff-Beard winter equation, depending on whether they were in a shaded or sun exposed microclimate, to estimate the PMI of the remains. The winter Marhoff-Beard equations were used in this instance because the degree of decomposition observed in each image indicated that the date of death of each set of remains was either relatively close to the 'date of discovery' in winter 2016, or the remains exhibited area of mummification or desiccation, which is the major criterion for selecting the winter equation (as outlined above in Section 7.2.1). Details of the environment in which the remains were found (i.e. shaded, sun exposed) were provided with the images, enabling the correct microclimate equation to be used in each instance.

The following table (Table 7-4) compares the known PMI with the predicted PMI generated when the Marhoff-Beard formula is applied.

Table 7-4 Comparison of the known PMI with the predicted PMI found when applying the Marhoff-Beard formula for pig remains deposited in the winter.

Pig	Decomposition stage	True PMI (days)	Predicted PMI (days)	Error in days[*]
1	Early	15	20	+5
2	Early	18	14	-4
3	Early	35	36	1
4	Early	45	38	-8
5	Early	28	30	+2
6	Advanced (desiccated)	122	153	+31
7	Advanced (desiccated)	112	49	-63
8	Advanced (desiccated)	97	52	-45
9	Advanced (desiccated)	122	105	-17

[*]The *error in days* is expressed as (–) the formula underestimated the PMI by the number of days listed. The *error in days* is expressed as (+) the formula overestimated the PMI by the number of days listed.

It can be observed from Table 7-4 above, that the Marhoff-Beard method can estimate the PMI of remains, with reasonable accuracy, when they present in the early decomposition stages. When the Marhoff-Beard formula was applied to the remains found in the early stage of decay, the largest error found during this time period was when the method underestimated the PMI by 8 days. This level of accuracy was not found when the three currently published methods were applied in the present study where it was found the currently published methods could estimate the PMI with an error as large as 20 days for the same early decomposition time period (Section 5.5.4, 5.7.3).

Once the remains desiccated in the advanced decomposition stage, the Marhoff-Beard method failed to accurately estimate the time since death. It is well accepted that once remains enter the advanced decomposition stage the ability to determine the PMI becomes increasingly difficult, more so, when remains have desiccated or mummified [22, 35, 53, 87, 121]. This may explain why the accuracy of the Marhoff-Beard formula is compromised at this time point.

7.3.2 Validating the summer equation on a porcine model

Table 7-5 represents the various climatic data collected on the 'date of discovery' of the remains, the observed degree of decay of each carcass and the known PMI of each set of remains at the time of the 'date of discovery'.

Table 7-5 Summary table of climatic values, total percentage of decay and known PMI of validation group of pig carcasses from summer 2013 and summer 2014.

Pig	% decay	Rainfall (mm)	Wind speed (km/h)	Humidity (%)	Date of Discovery	True PMI (days)
1	5	0	0.1	64.12	17/01/2013	2
2	30	0	0.1	69.59	25/01/2013	9
3	75	0	1	73.74	08/02/2013	25
4	45	1.4	1	92.78	22/01/2014	7
5	96	2.6	1	96.59	27/02/2014	44

Using the data outlined in Table 7-5, these values were entered into the appropriate Marhoff-Beard summer equation, depending on whether the remains were in a shaded or sun exposed microclimate as observed in the images, to estimate the PMI in days for the above carcasses. The summer Marhoff-Beard equations were used in this instance because the degree of decomposition observed in each image indicated that the date of death of each set of remains was either relatively close to the 'date of discovery' in summer 2013 or 2014, or the remains exhibited skeletonisation to the whole or part of the remains, which is the major criterion for selecting the summer equation. Details of the environment in which the remains were found (i.e. shaded, sun exposed) were provided with the images, enabling the correct microclimate equation to be used in each instance.

Table 7-6 compares the known PMI with the predicted PMI when the Marhoff-Beard formula is applied.

Pig	Decomposition stage	True PMI (days)	Predicted PMI (days)	Error in days[*]
1	Early	2	2	0
2	Early	9	12	+3
3	Early	7	3	-4
4	Advanced-skeletonisation	25	16	-9
5	Skeletonisation	44	1	-43

[*]The *error in days* is expressed as (-) the formula underestimated the PMI by the number of days listed. The *error in days* is expressed as (+) the formula overestimated the PMI by the number of days listed.

When the Marhoff-Beard summer formula is applied, the algorithm performed best when the carcasses were in the early decomposition stage, as was seen with the winter Marhoff-Beard equation. When remains were in the early decomposition stage the largest error found using this formula to estimate PMI was 6 days. As decomposition progressed into the more advanced stages and the remains began to skeletonise, the Marhoff-Beard method underestimated the PMI of the carcasses. This result was also found when applying the Marhoff-Beard winter formula to a porcine model.

Throughout the course of the present study, soft tissue decomposition was observed to occur rapidly during the summer months and in a short period of time the remains became mostly skeletonised (see Chapter 4). Given the high degree of decomposition observed within a short period of time, this may explain why the Marhoff-Beard method underestimated the PMI when a substantial amount (e.g. more than 75% as seen in Table 7-6) of soft tissue decay is observed.

7.4 Validating the new method on a human model: retrospective study

To determine if the Marhoff-Beard method could be transferred to human remains and still accurately estimate the PMI of remains and to evaluate its replicability, photographic images of decomposed human remains with a known PMI were obtained and tested.

Figure 7-3 demonstrates the division of a human body to assign a percentage of soft tissue decomposition to each individual body region. The same method using the 'Rule of Nine's' used in the scoring protocol from porcine remains (Chapter 7.2.1) was used on the human remains.

Anterior   **Posterior**
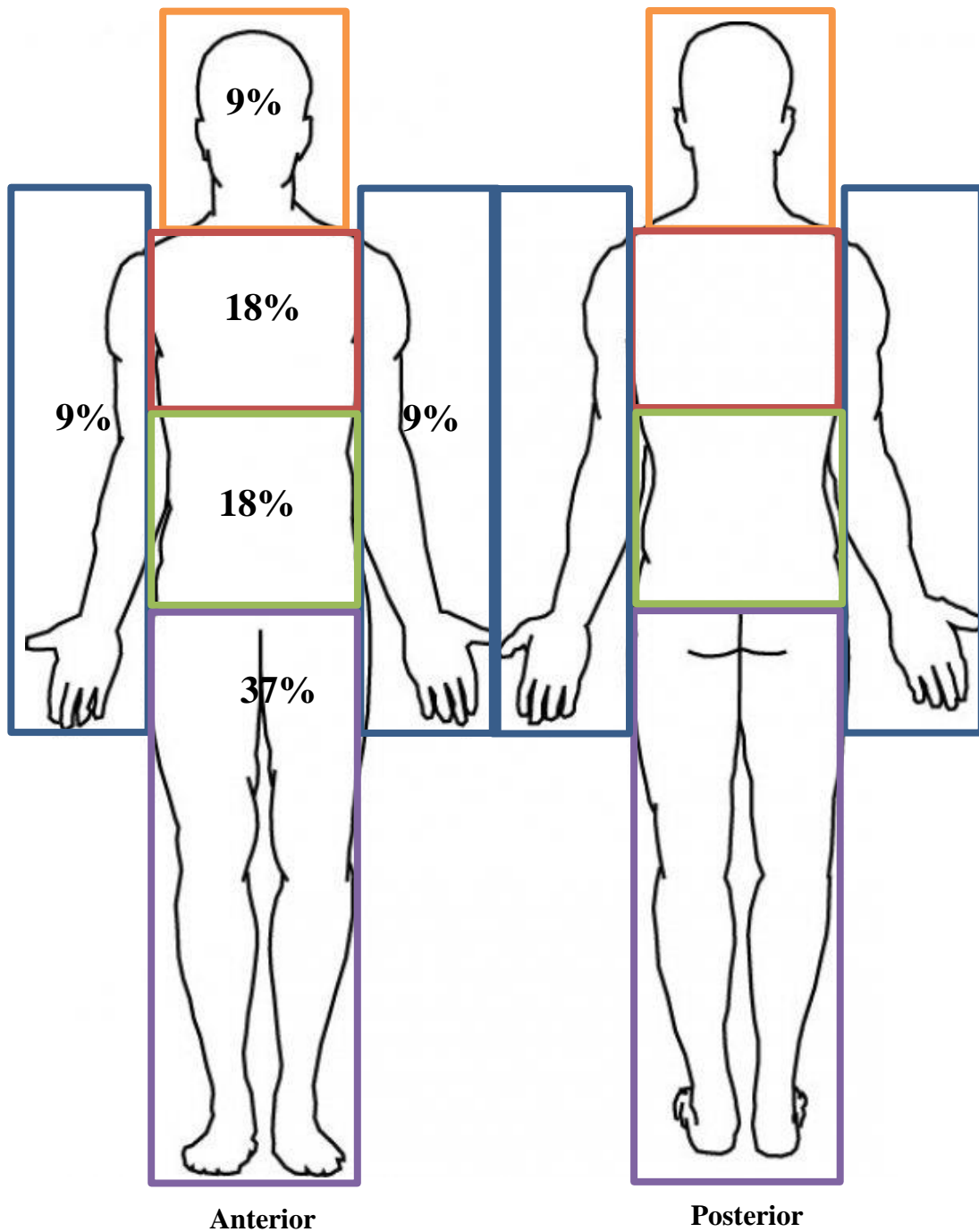
Figure 7-3 Division of a human body to estimate the percentage of decomposition that has occurred. ORANGE is the head and neck region, BLUE is the arms, RED is the thoracic region, GREEN is the abdomen and PURPLE is the legs, pelvic and gluteal region. These percentage values are the same whether the body is viewed from the anterior or the posterior aspect.

Each donor used in the present study had died of natural causes at varying times throughout 2016 (see Table 7-7) and were placed at the Australian Facility for Taphonomic Experimental Research (AFTER); a research site in Yarramundi, NSW, located approximately 14.9km from the current study location at Western Sydney University, Hawkesbury campus. Remains were placed in a low lying grass and soil area to naturally decompose on an environmental surface that is common throughout the Western Sydney region. This surface is comparable to the research site used in the current study and to the research site where the alternate summer and winter pig remains (as used for the validation of the Marhoff-Beard method) were located.

Photographs of decomposed human remains were used to evaluate the effectiveness of the Marhoff-Beard PMI formula when it is applied retrospectively, as photographs may be provided to a forensic anthropologist for a PMI determination rather than an "on scene" evaluation.

The following section presents the results of the validation study of the Marhoff-Beard formula for PMI determinations when applied to a human model. Table 7-7 represents the various climatic data collected on the 'date of discovery' of the remains, the observed degree of decay of each body and the known/true PMI of each set of remains at the time of the 'date of discovery'. Once again, date of death was not revealed to this author until scoring had been completed.

Table 7-7 Summary table of climatic values, total percentage of decay and known PMI of validation group of human remains from summer and winter 2016.

Body #	% decay	Rainfall (mm)	Wind speed (km/h)	Humidity (%)	Date of Death	Date of Discovery	True PMI (days)
1	0	0	30	75.9	27/07/2016	29/07/2016	2
2	40	1.2	57	74.6	27/07/2016	29/09/2016	44
3	20	0	33	60.1	18/11/2016	26/11/2016	7
4	60	0	44	75.5	20/04/2016	07/07/2016	77
5	80	0	44	75.5	02/02/2016	07/07/2016	155

Using the data outlined in Table 7-7, these values were entered into the appropriate Marhoff-Beard summer and winter equations according to their degree of decay, signalling that time of death was close to the known discovery date and/or, mummification or skeletonisation was observed at the 'date of discovery'. Details of the environment in which the remains were found (i.e. shaded, sun exposed) were provided with the images, enabling the correct microclimate equation to be used in each instance.

Table 7-8 compares the known PMI with the predicted PMI, found when the Marhoff-Beard formula is applied retrospectively to human remains.

Table 7-8 Comparison of the known PMI with the predicted PMI found when applying the Marhoff-Beard formula to human remains retrospectively.

Body #	Decomposition stage	True PMI (days)	Predicted PMI (days)	Error in days[*]
1	Fresh	2	2	0
2	Advanced	44	43	-1
3	Early	7	17	+10
4	Advanced	77	51	-26
5	Skeletonisation	155	53	-102

[*]The *error in days* is expressed as (–) the formula underestimated the PMI by the number of days listed. The *error in days* is expressed as (+) the formula overestimated the PMI by the number of days listed.

The Marhoff-Beard summer and winter equations had varying levels of accuracy when applied to the decomposed human remains in the photographs. When the winter (shade) equation was applied to Body 2 who was approximately 40% decomposed, an accurate estimate of PMI was achieved. However, when the summer (shade) equation was applied to a set of remains that was only 20% decomposed (Body 3) the formula did not produce an accurate result despite Body 3 being less decomposed.

Once the remains were determined to be desiccated in the advanced decomposition stage and the body was approximately 60% or more decomposed, the method failed to accurately estimate the time since death. Once remains entered the later stages of decay, the Marhoff-Beard formulas for both summer and winter (shade)

underestimated the PMI. This result is consistent with the findings from the porcine validation study.

Studies have found there may be ambiguity associated with identifying some morphological features from a decomposed body in photographs and the lack of context in photographs as opposed to what the investigator observes at the post-mortem scene [24]. Therefore, the Marhoff-Beard method for PMI estimations underwent further validation testing on a human body at the post-mortem scene from the beginning to the end of the decomposition process to evaluate its accuracy as a PMI method and its effectiveness when applied on the scene by the forensic anthropologist. These findings are reported below (Chapter 7.5).

7.5 Validating the new method on a human model: longitudinal study

To further test the accuracy and replicability of the Marhoff-Beard method at the scene of a body, a donated human body was observed from the beginning to the end of the decomposition process at AFTER. The male donor used in this study died on January 19 2017 and was placed at the research facility on January 25th 2017. The donor was refrigerated in the time period from death to placement at the site, potentially inhibiting the decomposition process.

The soil surface at AFTER was similar to the soil surface used for placement of all other pig and human remains in this study. The donor was positioned in the shade under the canopy of trees and covered with a metal cage (similar to Figure 2-3; Chapter 2.2) to prevent large vertebrate scavenging.

Table 7-9 presents the various climatic data collected on the 'date of discovery' of the remains, the observed degree of decay on each sampling day and the known/true PMI at the 'date of discovery'.

PMI	Date of Discovery	% decay	Rainfall (mm)	Wind speed (km/h)	Humidity (%)
2	27/01/2017	1	0.4	33	60
6	31/01/2017	5	0	56	29
8	02/02/2017	15	20.6	33	52
10	04/02/2017	15	0	20	79
13	07/02/2017	20	0	35	93
19	13/02/2017	40	0	43	20
21	15/02/2017	50	0	33	41
29	23/02/2017	60	0	31	31
42	09/03/2017	70	0	41	57

Using the data outlined in Table 7-9, these values were entered into the Marhoff-Beard summer shade equation as this set of remains was located in a shaded microclimate. Table 7-10 compares the known PMI with the predicted PMI, found when the Marhoff-Beard formula is applied. The predicted PMI determined using the Vass [3] and Megyesi et al. [1] formulas are provided for comparison.

Table 7-10 Comparison of the known PMI with the predicted PMI found when applying the Marhoff-Beard formula to human remains in the longitudinal study.

PMI	Predicted PMI (days)	Error in days[*]	Vass PMI (days)	Megyesi PMI (days)
2	1	-1	1	3
6	4	-2	6	5
8	7	-1	14	6
10	6	-4	10	6
13	17	+4	22	8
19	24	+5	29	10
21	36	+15	43	15
29	42	+13	86	15
42	43	+1	86	37

[*] The *error in days* is expressed as (–) the formula underestimated the PMI by the number of days listed. The *error in days* is expressed as (+) the formula overestimated the PMI by the number of days listed.

When applying the Marhoff-Beard summer equation to a human body that has been observed longitudinally, the formula performed well, producing relatively accurate PMI estimates up to when the body was approximately 40% decomposed which was observed by Day 19 post-mortem. Once again, the Marhoff-Beard formula produces the best PMI estimates through to the beginning of the advanced decomposition stage, however, once remains desiccate or mummify, the accuracy of the formula is compromised. This is a consistent finding that was discovered when the Marhoff-Beard formulas were tested on winter deposited pig carcasses, summer deposited pig carcasses, and when applied retrospectively to winter and summer human remains. The Marhoff-Beard method better estimated the PMI across more time points when compared to the PMI prediction by the Vass [3] and Megyesi et al. [1] formulas.

In the three abovementioned validation studies, it was found that once a set of remains reached desiccation, the Marhoff-Beard formula would underestimate the PMI. In the longitudinal human study, however, the formula was found to overestimate the PMI at this point. Potential reasons for this will be discussed below.

7.6 Discussion

A new method for estimating the PMI of decomposed remains in Greater Western Sydney was created with positive outcomes. Validation of this new Marhoff-Beard

method for determining PMI indicates this method can determine the PMI in a Western Sydney winter and summer climate and results were comparable when it was applied to both human remains and pig carcasses. Throughout the fresh and early decomposition stages, the Marhoff-Beard method demonstrated accurate PMI determinations during both seasons and in both humans and pig models. It is likely it performed best during these stages as decomposition of a body predominantly occurs internally during this time and perhaps it is not significantly impacted by external taphonomic factors such as microclimate and seasons. Hayman and Oxenham [100] support that decomposition is difficult to accurately determine after fourteen days as internal organ decomposition becomes too variable. As decomposition progresses, the body is more heavily reliant on the multitude of external taphonomic variables which may explain the inaccuracies in the PMI estimate found in the later decomposition stages, making it difficult to ascertain a correct PMI [100]. The ability of the Marhoff-Beard method may also have become limited once remains entered the advanced decomposition stage because the decomposition process slowed so significantly when the remains mummified or desiccated. This is a common issue with methods for PMI determinations [3].

The limited successes found with PMI methods, such as the Marhoff-Beard method, when remains enter the advanced decomposition stage suggests that the use of the morphological changes occurring in decomposition can only be used up until active decay ceases. Further investigation into the later stages of decay should be undertaken to determine what variables are affecting decomposition. Alternatively, a multidisciplinary approach may be required to produce accurate time since death determinations, for example using DNA degradation [122-124], degradation of bone products [5, 6, 125-128], change in the botanical products surrounding a body [129-131] or change in microbial composition of a body overtime [36, 132, 133].

Table 7-3 and Table 7-4 demonstrated that three sets of remains with the same degree of decay (15%) but different known PMI values can produce different, but accurate, predicted PMI's. This change is due to the change in weather data, suggesting that despite a lack of change in the degree of decay over different time periods, the method can still produce a different estimate of PMI based on the change in weather data alone. This was not found when applying the Megyesi et al. [1] and Marhoff et al. [2] methods or the Vass [3] formula, which produce the same estimate

of ADD/PMI. This is a unique characteristic of this method and clearly shows the importance of creating geographically specific methods for estimating PMI.

In comparison to the three previously published methods tested in the present study, the Marhoff-Beard method performed more accurately overall despite failing to accurately predict the true PMI after the advanced decomposition stage, when taking into consideration the accuracy of the prediction and the consistency of this accuracy across time points, sample type and seasons. Success was not observed at any time point when the Megyesi et al. [1] method was applied, while the Marhoff et al. [2] method appeared more accurate during the advanced decomposition stages but these results were not comparable at similar time points was not observed between trials and carcasses. The Vass [3] method was successfully applied during Trial 1 (winter) but failed to accurately estimate the PMI for all other trials.

The Marhoff-Beard method was created from a porcine model but was successfully applied to human remains during the early decomposition stages without further amendment to the method. The method of scoring the degree of decomposition, using the 'Rule of Nine's', (Figure 7-1 and Figure 7-3) may have contributed to the accuracy found when the Marhoff-Beard method was reproduced. To the best of our knowledge, this study is the first of its kind to use the 'Rule of Nine's' in this context. Difficulties were encountered when applying the published methods [1-3] as the scoring protocol was considered to be ambiguous and required the investigator to 'best estimate' the degree of decay that has occurred or to 'fit' the observed state of decay into a category. The scoring criteria accompanying the Marhoff-Beard PMI method requires the anthropologist to make a visual assessment of each of the individual body regions in relation to its surface area contribution to the total body to make an assessment of how much decomposition has occurred to each area. By defining the surface area of each body region, it is believed this may reduce some of the ambiguity associated with making an overall full body assessment of the degree of decomposition. It should be noted however that the method of scoring is still subjective and does not entirely remove the subjectivity.

The success of the validation study of the Marhoff-Beard method for PMI estimations shows it is an accurate and reproducible method when applied to remains in the early decomposition stage in Greater Western Sydney. While it is known that it can be successfully applied in Greater Western Sydney, it cannot be assumed the

Marhoff-Beard method will work in other geographic regions because it was created for a specific environmental location. Further validation is required in Australian geographic regions outside of the Greater Western Sydney basin.

Chapter Eight

8 Summary and Discussion

The aims of the present study were threefold. The study first aimed to document the decomposition process of thirty-two pig (*Sus scrofa domesticus*) carcasses in an area of Western Sydney that has been largely overlooked with regards to decomposition studies in the disciplines of forensic anthropology and taphonomy. The purpose of this was to gain an understanding of how remains decompose in this unique climatic environment. This knowledge would consequently aid in improving PMI estimations for the Greater Western Sydney region. Secondly, the study aimed to evaluate the accuracy and applicability of three anthropological methods for estimating the PMI of remains, which have not yet been investigated in detail here in Australia. By validating these methods, the present study would determine the appropriateness of these 'universal' standards in time since death estimations in temperate Australia. Finally, should the three evaluated methods fail to produce accurate estimations of PMI, we aimed to develop a new quantitative method to narrow the estimations of PMI of decomposed remains, specific to the Western Sydney area. The objective was to narrow PMI estimations in the region from weeks and months to days.

Three hypotheses were proposed. Hypothesis 1 proposed the warm weather associated with the summer months would accelerate the rate of soft tissue decomposition, resulting in a much shorter PMI when skeletonisation is complete in comparison to the winter trials and reported research performed in colder climates and seasons. Hypothesis 2 postulated that due to the climate specific nature of the decomposition process, the currently published methods for PMI estimates would incorrectly estimate the PMI or ADD of remains in the current study, suggesting these methods should not be applied in future estimations of PMI for remains found located in the Western Sydney region of eastern Australia. The third hypothesis proposed the newly created Marhoff-Beard method would produce more accurate predictions than those methods that have previously been developed internationally. Thus, this would provide a more appropriate model to use in a forensic setting to narrow down the time since death of remains found in the Greater Western Sydney

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area, with the potential to use this model across all temperate Australian climates. A summary of the results of the study that address each of the above aims is presented below:

Aim 1: To examine and document the decomposition process of pig carcasses, as analogues for human remains, and provide information on how the summer and winter climates of the Western Sydney region impact decay rates.

Decomposition rates, disarticulation sequences and soft tissue changes were observed on a total of thirty-two pig carcasses over a two year period, which encompassed two winter seasons (2014 and 2015) and two summer seasons (2014/15 and 2015/16). The literature states decomposition rates are climate and seasonally dependent [9, 65] and was evident during this project that there were major differences observed between the seasons. The behaviour of the winter carcasses across both trials can be summarised as follows:

- ❖ Decomposition was very slow for the winter carcasses and regularly stalled over the course of the winter trials.
- ❖ Remains only entered a partial bloated state and demonstrated a blue green discolouration on the abdomen, by approximately Day 10. Carcasses developed a dark green-black discolouration at the end of the early decomposition stage.
- ❖ The head and neck region of the carcasses was observed to have begun decomposing first, particularly at the mouth, followed by the limbs and finally the trunk.
- ❖ All winter remains mummified with no exceptions.
- ❖ The bones of the feet were found to disarticulate first, beginning with the phalanges, metatarsals then tarsals, followed by the disarticulation of the legs and jaw (mandible).
- ❖ Adipocere was only observed during the winter trials and was first observed as early as Day 13.

In comparison, the summer carcasses displayed:

- ❖ A much more rapid rate of decay.

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- ❖ Remains entered a fully bloated state and demonstrated a green-black discolouration simultaneously to bloating, at approximately Day 3. Once active decay commenced, a maroon-golden brown discolouration was observed when the skin began to slip and soft tissue decomposition began. This was not observed in the winter trials.
- ❖ Similar to the winter carcasses, the soft tissues of the head and neck began decomposing first, followed by the limbs, then the trunk in the summer carcasses.
- ❖ All summer remains skeletonised by Day 27, with no exceptions.
- ❖ The pattern of disarticulation of the bones was observed to be the same as the winter carcasses. It was found the phalanges, metatarsals, tarsals, legs and mandible disarticulated first, followed by the ribs and vertebral column.

Aim 2: To evaluate the accuracy and replicability of three currently published methods in the literature that have been proposed to accurately determine the PMI of remains.

Three previously published methods of determining the PMI from the degree of soft tissue decomposition were tested on thirty-two carcasses. Two of these methods were developed internationally and claim to be universally applicable [1, 3], while the third method was developed locally on a porcine model.

As Chapters 5 and 6 demonstrated, these published methods were not optimal for the current experimental conditions and environment. When the Megyesi et al. [1] method for ADD estimation is applied to remains in the Western Sydney region, it was found that for those carcasses deposited in the winter the method dramatically underestimated the true ADD (Section 5.5.4). Whereas for those carcasses deposited in the summer, the method overestimated the ADD and PMI (Section 6.5.4). Difficulties were found when applying the scoring protocol accompanying this method. The inaccuracy of this method increased as decomposition progressed. This is a common limitation of this method where many studies report erroneous PMI predictions once decomposition stalls [24, 53].

Through the validation study of the Marhoff et al. [2] formula it was determined the method underestimated the ADD in the winter trials (Section 5.7.3) but overestimated the ADD in the summer trials during the early decomposition stage

(Section 6.7.3). However, unlike the Megyesi et al. [1] method, this method performed better as the remains progressed through the advanced decomposition stage. The overall inaccuracy of this method may be attributed to the use of the original scoring protocol designed by Megyesi et al. [1] which was designed on humans but applied to pigs in the present study. While the equation was refined from the original method, the scoring model was not altered as such it should be determined if the accuracy of this method is improved when a new scoring protocol, made specific for the decomposition changes observed in Western Sydney, is created.

The Vass [3] formula performed differently to the other two methods tested in that it overestimated the PMI in the winter trials (Section 5.9.2) and underestimated the PMI of remains in the summer trials (Section 6.9.4). It performed better during the winter trials than the summer trials. The formula assumes the greater the percentage of soft tissue decomposition observed is related to a greater number of days passing in the PMI, i.e. it fails to identify the rapid onset of decay when remains are exposed to warmer conditions. The inaccuracy may be attributed to the constants (moisture and ADD) which make up the variables in this formula and they may not reflect the values of these variables in the Western Sydney region.

It was clear from the results of this analysis that the development of a new method that accounted for local climatic conditions was warranted.

> *Aim 3: To develop a new quantitative method to narrow the estimations of PMI of decomposed remains, specific to the Western Sydney region from weeks and months to days.*

A new formula for estimating the PMI of decomposed remains was created for the Greater Western Sydney region. To accompany this new Marhoff-Beard formula, a new method for quantifying decomposition was created based on the 'Rule of Nine's' method of estimating a body's surface area [120]. If the remains are observed to be in the early decomposition stage, the seasonally specific Marhoff-Beard equation can be applied depending on the current season when remains are discovered. If the remains are mostly mummified, the winter equation should be used and if the remains are mostly skeletonised, the summer equation can be used. This is

because in the present study we observed all winter remains to mummify and all summer remains to skeletonise without exception in the Western Sydney region. If the microclimate (sun or shade) is known or can be extrapolated from the photographs of the body, the microclimate specific Marhoff-Beard equation can be applied.

As demonstrated in Chapter 7, throughout the fresh and early decomposition stages the Marhoff-Beard method performed consistently well across the summer and winter seasons. The Marhoff-Beard formula and scoring protocol was developed of a pig model but was successfully applied to the both sample species (pig carcasses and human remains) in the validation study. The accuracy of the Marhoff-Beard method did become limited once remains entered the advanced decomposition stage because the decomposition rate of the remains slowed significantly when they mummified or desiccated.

In comparison to the previously published methods, the Marhoff-Beard formula performed better overall despite its limitations in the later decomposition stages when examining the accuracy of the prediction and the consistency of the accuracy across time points, sample types and seasons.

8.1 Discussion

8.1.1 Decomposition rate and climate

It is relatively well accepted that the climatic variables of any given geographic region, are one of the greatest influences on the decomposition process and rate [9, 11, 12, 15]. The results of the present study demonstrated that remains exposed to warmer conditions, such as those experienced in the summer months in eastern Australia, will decompose at a much more accelerated rate than those remains that have been deposited in cooler climates or during the months of winter. This is comparative to findings in the literature which suggest the combination of increased rainfall, higher temperatures and greater humidity levels, such as experienced in the summer, will hasten the progression of the decomposition process as the colonisation of bacteria is optimal between temperatures of 25-35ºC [65, 75].

During the summer seasons, soft tissue decomposition was observed to continuously decompose exponentially before entering the skeletonisation stage. Skeletonisation

was reached within four weeks for all summer carcasses in the present study. This is similar to results found in previous studies which have demonstrated that when conditions are optimal, soft tissue decomposition will progress rapidly and skeletonisation can occur within four weeks of the PMI [9, 24, 72, 110, 115, 134]. The skeletonisation stage was characterised by extensive exposure of the bones of the ribs, legs, vertebral column and skull.

The cooler winter conditions of Trial 1 and Trial 3 greatly retarded the rate of decay. Exponential change in the decomposition process was observed during the winter trials followed by a plateau at the end of each stage before the carcasses progressed into the next stage of decomposition. The sixteen carcasses in the two winter trials of the current study, mummified and did not progress beyond the advanced decomposition stage, as classified by Galloway et al. [16]. Typically, mummification occurs out in the open or in shallow burials, but requires cool and dry conditions [135, 136]. Therefore, the wooded area the remains were placed under in the present study may have acted to keep the temperatures down and protected the remains from rain and other sources of moisture such as early morning condensation. Once the remains mummified they subsequently entered a period of stasis whereby decomposition ceased and the carcasses remained in a fixed state of decay. This pattern is consistent with previous research conducted in the winter both nationally and internationally [2, 13, 18, 22, 24].

During advanced decomposition, exposure of the skeleton was observed in the winter trials, albeit to a considerably lesser degree than observed in summer. In the winter trials only the bones of the legs and jaw became exposed before the remains mummified. This pattern is likely due to the smaller musculature present in the skull and legs of pigs compared to the trunk, thus, decomposing quicker than the larger muscles of the body [83]. The increased rate of decomposition during the summer trials when compared to winter may explain why the trunk was able to reach skeletonisation as well as the head and limbs, while only the areas with smaller musculature were able to reach the skeletonisation stage during the slower rates observed in winter [137]. The exposed bones of remains in the summer trials were observed to have a greasy black substance on the periosteum before the rainfall washed them clean. This greasy substance was not observed on the exposed bones in the winter trials and may be due to the rapid succession of soft tissue decomposition

in summer, leading to the liquefaction from the body covering the bones. This is in comparison to the winter trials, when the limited bone exposure was observed, it occurred much slower, possibly allowing the bones to dry simultaneously with exposure.

While there were differences in the rates of decay between the summer and winter trials of the present study, such as the pattern of bloating and discolouration, there were also some similarities between these carcasses during these two seasons. Most notably the sequence of soft tissue decay, and the disarticulation sequence of the joints and bones were found to have the greatest similarity between the trial seasons.

8.1.2 Pattern of bloating and discolouration

It was observed in the present study that that the pattern of bloating was similar across the seasons. It has been suggested previously by Megyesi et al. [1] that the limbs do not bloat. However, the limbs of all thirty-two remains in the current study presented with bloat (Chapter 4, Figure 4-3). This finding is consistent with the observations from a 2013 study in the Hawkesbury region, where the remains in this study also presented with bloated limbs during the early decomposition stage [26].

While the degree of bloating varied *between* the two summer and winter seasons, it was observed to be consistent *within* the seasonal specific trials. Remains only achieved a partial state of bloat in the winter trials, whereas, the carcasses in the summer trials entered a fully bloated state. This result was also found in a study in Texas, where the degree of bloat was also varied between the seasonal studies [24]. The timing of the post-bloat phase was comparable between the two summer trials and Ayers [37] study on the decomposition process of pig carcasses in Texas where all remains entered post-bloat on Day 4. It should be noted that while the degree of bloat was different between seasons, all regions of the body of the carcasses would bloat at the same time, i.e. the individual body regions did not bloat independently of one another.

The pattern of skin discolouration also differed between the two seasons studied. A blue-green discolouration was observed on the abdomen (Chapter 3, Figure 3-1b) throughout the two winter trials, however, this was not observed during the summer trials. Instead, during the summer trials, the remains first demonstrated a dark green-black discolouration, simultaneously to the onset of bloat, and once active decay

commenced, a maroon-golden brown discolouration was observed when the skin began to slip and soft tissue decomposition began (Chapter 4, Figure 4-6). The carcasses in the winter trials did not develop a dark green-black discolouration until they entered the later stage of early decomposition, progressing into the advanced decomposition stage.

The summer carcasses demonstrated extensive skin slippage in both trials and this may be why the maroon discolouration was only observed on the summer remains whereas only minimal skin slip was observed on the winter carcasses. The dark discolouration observed in the summer trials was obvious from approximately Day 3. It may be that the typical blue-green discolouration did appear on the carcasses during the summer trials but this was not observed at the time of sampling as the progression of decomposition and the resulting morphological changes occurred quickly. Enwere [112] also reported only observing a dark green-black discolouration on remains during their study and did not observe the typical blue-green discolouration.

8.1.3   Sequence of decomposition

Throughout each of the four trials undertaken in the present study, the head and neck region of the carcasses was observed to begin decomposition first, particularly at the mouth, followed by the limbs and finally the trunk was observed to have begun decomposing last. Previous national and international decomposition studies have reported similar results in their investigations into the sequence and pattern of decay [2, 20, 113, 114]. It is possible that the areas of the head and neck decompose first as there are fewer structures and the musculature is smaller within these regions when compared to those structures within the trunk and, therefore, the smaller the structure the less time it takes to decompose. The head region may also be the first region to decompose, as there are a number of access points to the body. These include facial orifices such as the mouth, eyes, ears and nose, which insects prefer to first colonise at as there is an abundant of food sources in these areas and the flies will lay eggs here ready for the larvae to hatch and take refuge in the warmth of the body [136]. Sutherland et al. [55] and Parsons [53] suggest the odour emitted from the mouth and nose of a carcass may attract the flies, resulting in colonisation of these areas first. This may explain the pattern of skeletonisation observed in the body regions during the summer and winter trials (Section 8.1.1).

8.1.4 Disarticulation sequence

Despite being an important consideration in taphonomic studies, the disarticulation sequence of remains is relatively understudied [19]. There was no seasonal difference in the disarticulation sequence of remains in the present study.

In Haglund's [83] investigation into the disarticulation pattern of the *human* skeleton, it was found that the bones of the hands and wrist disarticulated first followed by the mandible, cranium, lower legs, forearms, arms, and finally the bones of the trunk and pelvic girdle. He concluded remains disarticulate in order of the flexibility of the joint (i.e. degree of ligamentous binding). In the present study, the bones of the feet disarticulated first, followed by the legs, mandible, ribs and vertebral column. It was also observed during all four trials, that soft tissue first disappeared from the mandible, skull and facial bones, feet, legs and finally the trunk, a finding comparable to Haglund [83]. However, the results of the present study contradict a recent Australian disarticulation study [19]. Cameron and Oxenham [19] found the mandible, cervical vertebrae and ribs disarticulated first but the forelimbs and hind limbs took the greatest amount of time to disarticulate.

Haglund [83] concluded that the deposition environment of a body and the variables the body is exposed to within that environment, will greatly influence the pattern of soft tissue decay and skeletal disarticulation which may explain the differences observed between the present study and Cameron and Oxenham [19]. It also highlights a need for further research in different Australian environments to investigate in detail why these differences in disarticulation sequences occur.

8.1.5 Presence of adipocere

The presence of adipocere was also only observed in one of the trials undertaken; Trial 1 (winter). Adipocere was observed on three carcasses in this trial, all of which were located in the unshaded microclimate. Ideal conditions for the formation of adipocere include a warm, damp environment, whereas colder climates will inhibit its formation [70, 107, 138]. This is interesting as the ideal conditions for the development of adipocere on or around a body is more representative of the climate experienced in Australian summer months. This is also contrary to the ideal conditions for mummification, which as mentioned previously in Section 8.1.1, each carcass in Trial 1 and Trial 3 mummified.

The timing of the formation of adipocere occurred earlier than what is generally reported in the literature. Adipocere was first observed on Day 13 in Trial 1 (winter), however, studies show adipocere does not usually present until weeks or months after death. Vass et al. [45] reported adipocere did not first present until approximately Day 91 in their winter-spring study. However, Yan et al. [139] reported they first observed the formation some hours after death but it took several weeks for the body to present with a more developed formation. The timing and presence of adipocere is highly variable and is impacted by a multitude of variables. It has been suggested that while the environment the remains are present in is important in its development, it is the immediate microclimate of the body which is most influential and considerable variation in the presence of adipocere can occur between individuals deposited in the same area, as found in the present study [104].

8.2 Quantifying decomposition: Total Body Score and percentage of decay

The second aim of the present study was to apply the methods for PMI estimates developed by Megyesi et al. [1], Marhoff et al. [2] and Vass [3], using a pig model. Within these various methods was a means to quantify the observed decomposition state in order to apply the respective equations from these methods for estimating the time since death.

8.2.1 The use of a Total Body Score to quantify decomposition

As described in Chapter 2, both the Megyesi et al. [1] method and Marhoff et al. [2] method make use of the Total Body Score scoring protocol, as created by Megyesi et al. [1] (Appendix A). This scoring protocol is a modification of the Galloway et al. [16] categories and stages of decay where the researchers divided decomposition into four distinct categories: fresh, early decomposition, advanced decomposition and skeletonisation. These four categories were further divided into a number of commonly observed taphonomic changes and characteristics for the three body regions: head and neck, trunk, limbs. For each of these outlined characteristics or changes in each stage for each of the three body regions, a point value is allocated (Appendix A). The three scores, one for each body regions, are summated to generate a final score representative of the overall state of decay of the body referred

to as the Total Body Score (TBS). When the scores are summated the minimum TBS value remains can achieve is 3 (fresh remains overall) and the maximum TBS is 35 (dry bones overall).

The stages of decomposition, such as those outlined by Galloway et al. [16], Rodriguez and Bass [12] and Reed [21], have always been considered subjective but accepted to occur in a sequential order. It is the taphonomic changes or characteristics within these stages that do not appear in a relatively ordered fashion. Megyesi et al. [1] claim these characteristics appear continuously, hence the ability to create a standard scoring protocol. It became apparent in the current study that the soft tissue changes in the Megyesi et al. [1] model, were not always observed or they occurred out of order than what was described, making it difficult to assign an appropriate and accurate TBS to reflect the changes observed. For example, when examining the changes to the head and neck region of the remains, it was found the grey/green discolouration, brownish shades at the edges and caving in of the tissues of the eyes did not take place. Skin slippage for all three body regions was also found to occur only once bloating had subsided, whereas the scoring protocol suggests skin slippage should be one of the first decomposition changes to occur and before the body bloats [1].

As discussed previously (Section 8.1.2), Megyesi et al. [1] concluded limbs do not bloat, as such, this characteristic is not accounted for in the TBS protocol. However, this study along with previous research [24, 26, 140], observed all four limbs to bloat when the rest of the carcass or body bloated. In the current study, a red and golden brown discolouration appeared on each set of summer remains, toward the end of the early decomposition stage when the remains were actively decaying particularly on the trunk and limbs. A similar finding was discovered in Dabbs et al. [140] research yet this colour change is not represented in the scoring protocol, making it difficult for the researcher to apply a TBS value to these regions.

Megyesi et al. [1] acknowledge in their original study, that only human remains with a PMI of less than one year were used to create this method as soft tissue is rarely present beyond a PMI of one year in most temperate climates. The present study disputes this theory as in both winter trials, mummified soft tissue remained on the carcasses more than 18-24 months after the conclusion of the trials (results not shown). While the scoring protocol accounts for mummification, Megyesi et al. [1]

did not actually observe this decomposition state, instead, incorporating it into their scoring protocol based on how mummification occurs in non-desert regions of the United States [1]. This statement suggests that attempting to apply a TBS to mummified remains outside of specific regions of the United States may not be appropriate. As such, this may be a limitation of the TBS scoring protocol and its application to remains found in Australia.

Further difficulty was found when applying a TBS value to a body region. Megyesi et al. [1] recognize that in some cases, decomposition will not follow the defined sequence outlined in the scoring protocol. For example, they noted that they had observed drying of the extremities on a set of remains (assigned a point value of '4') had appeared before any discolouration (assigned a point value of '3') whereas the protocol suggests discolouration of the remains should occur before drying. In this instance, they recommend a point value should be assigned based on the earliest change i.e. a point value of '3', regardless of the order the changes present. The scoring protocol assumes that decomposition is sequential, therefore, the TBS is not a true representation of the actual changes observed.

Dautartas [72] reported issues with the Megyesi et al. [1] scoring model and found difficulties 'fitting' remains into the various stages outlined in the protocol, especially once remains mummified as there is no specific score to assign when remains are completely mummified because the protocol only accounts for partial mummification. This was also found in the present study. This likely led to an inaccurate TBS assigned to a set of remains which may have impacted on the accuracy of the ADD prediction. As such, the scoring protocol of this method is a limitation of this method.

A further limitation of the TBS protocol with regards to mummified remains is that mummified remains are unlikely to progress into the skeletonisation stage for years, if they progress at all. As a result, the same TBS is applied throughout this time period, subsequently producing estimates of ADD and PMI that do not alter from the current estimate at the onset of mummification, despite degrees and time continuing to accumulate. This was found in previous studies [24, 53] and in the present study for mummified and skeletonised remains, as the same TBS value was applied over many days which resulted in the same estimate of ADD being produced and irrespective of whether they had been exposed for a short time (that is, they

skeletonised early in the PMI) or over an extended period of time (Figure 5-7, Figure 5-10, Figure 6-7, Figure 6-10).

The scoring protocol associated with the Megyesi et al. [1] and Marhoff et al. [2] methods is highly specific in that it divides the body into three distinct body regions and outlines a series of decomposition changes generally found to occur at these regions. Given the difficulties found when attempting to apply a single TBS to a body region, this may mean that the scoring protocol is too specific as it does not allow the researcher to apply their own observations particularly if different changes are observed. This also means the opposite is true in that the scoring protocol then becomes too subjective as the researcher must interpret the protocol so it best fits their observations, leading to mistaken PMI estimations when applied to the overall method.

If the TBS scoring protocol is to be applied for either the Megyesi et al. [1] or Marhoff et al. [2] methods, the scoring protocol should be revised to include the observations bodies demonstrate within individual geographic regions, such as the Greater Western Sydney region. By making the scoring protocol specific for individual geographic or environmental regions, this may contribute to improvements in PMI estimates when these methods are applied.

8.2.2 Applying a percentage to quantify the degree of soft tissue decomposition

The Vass Universal PMI Formula [3] uses a cumulative percentage to quantify the degree of decomposition observed in order to calculate the PMI of remains. The method requires the researcher to evaluate the level of soft tissue decomposition and apply either a percentage range or a single decomposition value representative of the overall state of decay of the body. The minimum value that can be assigned is 0% (fresh, no decomposition yet occurred) and the maximum being 100% (complete soft tissue decomposition). Unlike the Megyesi et al. [1] scoring protocol, there is no description or explanation as to what changes or characteristics are used to evaluate the degree of soft tissue decomposition to express it as a numeric value. Instead, the Vass [3] method provides a table which correlates a percentage range with each of the four decomposition stages: fresh, bloat, decay and dry. When this table was followed in the present study, the range Vass assigned to each decomposition stage

did not accurately reflect the observed degree of decay in the current study. This may be explained by the overlapping decomposition stages observed in a single carcass throughout the four trials which is not reflected in the table. This scoring method [3] may better represent the decomposition process observed in Tennessee, where the method was originally developed, but should be used with caution in Australian climates.

The maximum percentage value assigned to remains in the winter trials in the present study was 70% (mummified remains, some bone exposure, loss of structural integrity, some soft tissue decomposed) and the maximum value assigned to the summer remains was 95% (dry bones, all soft tissue decomposed with some dried connective tissues remaining attached to the bones). The method was found to be particularly difficult to assign a value when only minimal changes in the soft tissue occurred making it difficult to assess increases in the change in decomposition at smaller increments such as 1 or 2 percent.

In order for this method to accurately estimate the PMI, Vass [3] outlines a series of specific criteria regarding the state of the remains. As part of the outlined criteria, Vass [3] notes:

❖ remains must not yet have skeletonised,
❖ the ADD has not exceeded 1285,
❖ soft tissues must be present,
❖ if the remains have mummified the tissues must still be soft and supple,
❖ the body should not have undergone excessive soft tissue trauma
❖ adipocere should not be present on the remains or at least very little present.

In the completed trials of this study, carcasses demonstrated hardened mummified tissues in the winter and skeletonised remains in the summer. These findings do not meet the criteria outlined in this method, meaning these carcasses should not have been scored. The method [3] is unclear as to what 100% soft tissue decomposition is if remains cannot be skeletonised and an explanation is not provided. This makes it difficult to assign a value to the observed taphonomic changes if explanations or descriptions are lacking from this method. This lack of clarity could lead the researcher to underscore or overscore the percentage of soft tissue decay when the above criteria are employed.

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Further issues arise when evaluating the level of soft tissue decay, as Vass [3] does not define whether the researcher should be assessing the outer soft tissues only, or, take into consideration the loss of soft tissues internally as well (i.e. the loss of biomass). It has been found in previous research that decomposition of the internal tissues can occur without the outer soft tissues decomposing [9], or, the outer tissues are showing fewer signs of decay. This observation may have implications in time since death estimates when applying the Vass [3] method, as the internal structures are not considered, despite them decomposing and time elapsing. While evaluation of the decomposition of the internal structures may not be possible in the field, perhaps this should be investigated at autopsy to better apply the method and increase the accuracy of the PMI estimate.

Vass [3] suggests that the percentage of decomposition that is to be applied to remains must be determined by a qualified and experienced investigator, preferably in collaboration with a forensic pathologist or anthropologist. He also notes a single value should only be applied when the researcher or investigator is confident in their assessment. Difficulty in estimating the degree of decay was found in the current study. Given that no particular definitions or explanations are provided as to what to estimate has decomposed (e.g. outer soft tissues only, internal decomposition), it makes it difficult to apply an appropriate value that accurately defines and depicts the observed state of decay. A lack of experience by the observer in determining the correct degree of decay is a potential limitation of this study, and should be further investigated as it may impact on the accuracy and applicability of this method.

Despite the potential difficulties in applying a single value to the degree of soft tissue decay from the perspective of a novice investigator, this author believes that the application of an overall percentage of soft tissue decomposition is a better alternative to a standard scoring protocol as it reflects real time observations of the remains and does not require the investigator to 'fit' the observed state of decay into a category, as seen in Megyesi et al.'s [1] scoring protocol. However, this could be further improved by dividing the body into regions and analysing these regions individually to determine an estimate of decay based on the ratio of that segment to the body as a whole. These values for the body segments/regions could then be summated to produce a total percentage of decay of the body, thus eliminating some of the ambiguity associated with how much an area of the body comprises in relation

to the entire body. This formed the basis of our third aim, discussed below in Section 8.4.

8.3 The application of current methods to estimate PMI in temperate Australia

For each of the three methods tested, statistically significant differences were found when comparing the predicted values to the known PMI and ADD in the present study (Chapters 5 and 6). The two internationally developed methods are designed to be universal predictors of the PMI contrary to geographical locations. However, the inaccuracies found when applying them to estimate the PMI of remains deposited in Australia, in particular the Western Sydney region where the present study was conducted, have many implications in forensic investigations when the PMI is unknown. In all cases of this study these methods largely underestimated or overestimated the PMI of remains, and ultimately, were determined to be too inaccurate to be used in future investigations.

Possible reasons for the discrepancies between the true PMI and the predicted PMI determined by these three methods may be due to the specific environmental variables influencing the decomposition process in Western Sydney. Two of the three methods evaluated in the present study (the Megyesi et al. [1] and Vass [3] methods) were developed internationally in the United States, therefore, the values and variables used in those studies to create the methods may only be specific to those regions.

The below sections describe in further detail the applicability of the Megyesi et al. [1], Marhoff et al. [2], and Vass [3] methods.

8.3.1 The Megyesi et al. ADD method

When the Megyesi et al. [1] method for ADD estimation was applied to remains in the Western Sydney region, it was found that for those carcasses deposited in the winter the method dramatically underestimated the true ADD and, subsequently, the PMI of the remains. For those carcasses deposited in the summer, the method overestimated the ADD and PMI. The degree of over and underestimation of the ADD and PMI was not consistent throughout the course of the study, making it

difficult to ascertain what variables are most likely impacting or influencing the decomposition process when this occurs.

Despite it being widely accepted that the process of decomposition is climate and geographically dependant [12, 15-18]. Megyesi et al. [1] acknowledge in their original study that their formula does not include other important taphonomic variables such as rainfall, humidity and scavenging for example, despite the understanding that these variables significantly impact decay rates. They determined ADD alone could explain the variation in decomposition without the need to include any other variables. Climatic conditions experienced here in temperate Australia are vastly different from climatic conditions in the mid-west United States where this method was developed [141, 142]. Given the lack of accuracy found in the current investigation when applying the Megyesi et al. [1] formula, perhaps further taphonomic variables, unique to this Australian environment, are influencing the decay process and, as such, cannot be accounted for in this method. Further studies should be undertaken to determine what these variables are and how they affect the decomposition process to better PMI estimates in the future.

The method [1] appeared to become more accurate as decomposition advanced in the present study. Once remains mummified or skeletonised, the same TBS value was assigned multiple times resulting in the same ADD being estimated each time over that period. Degrees continued to accumulate causing the predicted and true ADD values to ultimately converge, thus, appearing as if the method becomes more accurate. Similar results were found in Parsons 2009 study [53] and they too, concluded that the prolonged periods of stasis in the decomposition process as a result of the effects of a winter climate, impacted positively on the accuracy of this method, allowing the true ADD temperatures to 'catch up' to the prediction and therefore improve the PMI calculation. However, overall we concluded in the present study, that with the exception of two time points across the PMI when a TBS is *first* assigned, this method did not perform with any accuracy.

Finally, Megyesi et al. [1] created the ADD method from photographs of human remains and the method was designed to be applied to human remains for PMI determinations. We evaluated the accuracy of this method on a porcine model which may have contributed to some of the inaccuracies found when applying this method in the present study. This finding has been found previously by Keough et al. [119]

where they suggested perhaps a modified scoring protocol may be required if the Megyesi et al. [1] method is to be tested on pig remains.

Megyesi et al. [1] provide the suggestion that future research should investigate more defined locations to create and develop more specific equations for those environments. The findings in this study support Megyesi et al.'s [1] statement, and this research suggests that the original formula is not applicable in temperate Australian conditions and geographic specific algorithms and methods should be developed to better narrow PMI determinations.

8.3.2   The Marhoff et al. formula

The Marhoff et al. [2] formula was created in 2013 after a preliminary study evaluated the applicability of the Megyesi et al. [1] ADD method. The 2013 study [26] found the original method [1] underestimated the ADD of remains, as such, the equation was refined and made specific to the region while still following the original scoring protocol. The present study evaluated the newly refined ADD equation and found that it too underestimated the ADD that carcasses were exposed to in both the summer and winter trials, however, it was found to be more accurate than predictions generated by the original Megyesi et al. equation [1].

This method was originally created from a small sample size of only four pig carcasses used as proxies for human remains. Developing a formula from a small sample size is a limitation of this method as few decomposition sequences would be observed and, subsequently, be accounted for in the method. It did produce a positive result in the present study in that it achieved its purpose by narrowing PMI predictions for the region when using the Megyesi et al. [1] formula. Although it underestimated the ADD and PMI in the winter seasons, it did perform better during these winter trials than in summer. As it was originally created from data collected in a trial completed during the winter months of 2013, this result is not surprising and also suggests that seasonally specific methods could potentially be created by using a more robust sample size and a more comprehensive investigation.

Furthermore, it was found that all remains used in the 2013 trial reached mummification and did not progress beyond this stage [26]. The carcasses had been scored TBS values equivalent to the advanced decomposition stage for most of the duration of the 2013 original trial. It is possible the method better estimates the ADD

in the later decay stages, as found in the current study, as it was created from desiccated remains. It is difficult to draw definitive conclusions however, as this formula has not yet been validated in any other study.

When comparing the results of the linear mixed model for both winter trials to determine if the Marhoff et al. [2] method produces more accurate results when obtaining temperatures at the site of the remains or obtaining temperatures from the local weather station (BoM), the method produced marginally more accurate estimations of ADD when using temperatures recorded by the local BoM weather station. This is a positive result as forensic investigators would likely only have access to local weather station data and not data from the post-mortem scene.

Based on the results of the validation of the Marhoff et al. [2] method in the present study, it appears the method performed best during the advanced decomposition stage. When certain TBS values were first assigned to the remains, the method could accurately predict the ADD to within one to two days of the true ADD and PMI in some cases (see Chapter 5; Table 5-4,). Again, it should be cautioned that although accuracy was found in certain trials with the above TBS values, this was not the case for each of the four trials and for each carcass and it cannot be assumed the same accuracy will be found in future investigations.

While the equation was refined to better suit the conditions of the Greater Western Sydney region, it was found that the method still did not precisely estimate the correct ADD the remains were exposed to. To accompany the equation, Megyesi et al. [1] created a scoring protocol to produce the TBS value. This same scoring protocol was employed in the Marhoff et al. [2] method without alterations. Perhaps given the inaccuracies found when applying this method in temperate Australia, the scoring protocol should also be adjusted to better suit the taphonomic changes demonstrated by remains in this environment. Refining the scoring protocol and the TBS scores assigned, may improve the accuracy of this model. Future studies should investigate if a more specific scoring protocol will improve this method.

The Marhoff et al. [2] and the Megyesi et al. [1] formulas do not contain any 'real time' taphonomic variables that may influence decay rates, such as, rainfall and temperature. The method assumes that the formula incorporates temperature based on the constants provided but does not include the actual average daily temperature. It was found in the current study, that temperature was not a statistically significant

variable (Section 7.2), yet this method is based around the theory that temperature is the *most* influential variable concerned with decomposition rates. This finding suggests that perhaps because this method does not incorporate any further variables it cannot accurately estimate the ADD and PMI of remains in this region, as there are other environmental factors influencing the decay process.

This is the first study validating the use of the Marhoff et al. [2] regression equation. As there is no data collected from other studies, it makes it difficult to compare the results found in the current study and draw conclusions. Based on this study alone, it was found this method needs further refinement if the ADD method and concept is to be used in temperate Australia.

8.3.3   The Vass Universal PMI formula

In brief, the present study determined the Vass [3] formula generates rather varied results in that it either largely overestimated the PMI or it dramatically underestimated the PMI, within any given season. This differs from the previous two methods where the under estimation and overestimation of the PMI was confined within each season. This result is different to Cockle and Bell [143] where it was found in their 2015 study evaluating the Vass [3] method using decomposed human remains in Canada that the method overestimated the PMI in warmer conditions but in cooler conditions the formula underestimated the time since death [50]. In the present study, the Vass [3] method performed better at estimating the time since death of remains located in the shaded microclimate, and accurately determined the PMI (to within 3 days of the true PMI) for a limited number of carcasses in Trial 1 (winter) and Trial 4 (summer).

This formula does incorporate specific environmental variables such as humidity levels and the average daily temperature; however, it also incorporates two constants that have been derived from studies undertaken in the United States, specifically at the Anthropological Research Facility in Knoxville, Tennessee. One of these constants is for the average amount of moisture present in the air at any point in time and the other is the average ADD in which decomposition is said to cease. Given that real time variables are used in this formula, thus, giving it the potential to be used universally, perhaps it is the constants which impact on the accuracy. It can be postulated that the value for the degree of moisture Vass has derived can differ from

region to region and does not accurately represent the degree of moisture contributing to the decomposition of remains in temperate Australia. Cockle and Bell [50] also suggest that the value calculated by Vass [3] may not be constant for all geographical locations. Further studies should be undertaken to determine the empirical value for moisture in this region. Carter et al. [144] postulate that moisture levels are one of the most important variables governing the decay process and therefore this variable should be represented accurately if this method for PMI determinations is to be used.

The standard ADD that has also been derived by Vass may also not represent the true decay process observed in temperate Australia. It was found in the present study that remains appeared to cease decomposition, on average, after 980 ADD in the winter and after 1069 ADD in the summer, however, Vass [3] theorises that decomposition should cease at approximately 1285 ADD. Given that ADD and moisture levels are key components of this formula, further studies calculating these values for temperate Australia should be undertaken and the algorithm refined to reflect temperate Australian conditions.

As mentioned above this formula makes use of taphonomic variables such as temperature and humidity to estimate time since death. Vass [3] proposes that to obtain temperature data, one could use the average daily temperature on the day the remains were discovered and input that value into the equation, or, the investigator can use the average daily temperature of the ten days prior to the date of discovery. Whether the temperature data from the date of discovery is applied or whether the average daily temperature over a 10-day period is applied (results not shown), neither of these improved the PMI estimation. As it was found in the current study that this method both over and under estimated the PMI, perhaps this method does not include all the influential factors affecting decomposition in this unique region or it may simply not include the most *influential* variables affecting the decay process. It was found during the course of the present study that the temperature variable was not statistically significant in the decomposition process, but rather the environmental factors humidity, rainfall and wind speed were more influential in explaining the variability in the rate of decomposition, and perhaps a method using these variables would be more applicable in Australian climates.

As discussed previously (Section 8.2.2), there are difficulties in applying one single value to the degree of decay and this may also have an impact on the accuracy of this model. If one miscalculates the percentage of soft tissue decomposition it can result in erroneous PMI predictions. To reduce the level of error that is found when applying a single value to the observed degree of decomposition, Vass suggests applying a percentage range. For example, overall decomposition could be scored as 25-35% as opposed to a single value of 25%. However, the equation cannot be applied by directly inserting this range into the equation and therefore, the equation must be applied twice in order to generate a PMI range using the upper and lower values of the decomposition range. Perhaps to increase the accuracy when applying a single value to a set of remains, greater explanation of when certain values should be applied may make it more appropriate. For example, what taphonomic changes would likely or commonly be observed when a body is x% decomposed. Although the decomposition process is variable, an explanation or description like this could narrow and refine the percentage of decay an investigator applies if this information is available.

In addition to the variables outlined in the formula, Vass [3] recommends that to assist in determining the degree of decay a body has undergone, one could conduct additional tests to verify the presence of volatile fatty acids. This suggests an issue with the method of quantifying decomposition as a percentage of decay, if it is recognised that further, more analytical testing needs to be undertaken. Or perhaps this method is more applicable as a presumptive test on scene with further confirmatory tests required.

Finally, the original protocol created by Vass [3], was developed based on a human model, however, our study aimed to validate this method on a porcine model. While there are some notable similarities between humans and pig carcasses, there are anatomical differences between these subjects (for example, stature and length of limbs, fat layer). As the Vass [3] formula was created from and to be used on a human model, our use of pigs as an analogue for human remains may be a limitation of this study and may have contributed to the inaccuracies found when estimating the PMI.

The formula was created to be a 'rough estimate' required by forensics and police investigators to begin their investigation. The use of this method would then be

superseded by more analytical methods of determining PMI following lab analyses. These more analytical methods for determining PMI would then be used in any future legal proceedings. The estimates of PMI generated in the current study are too inaccurate to be considered 'rough estimates' and would not narrow a time frame for which investigative personnel could begin their investigation.

Like with the Marhoff et al. [2] equation, no other study has evaluated the applicability of the Vass [3] method in Australia. As such, it makes it difficult to compare results on a local or national scale. Based on this study alone, it was concluded this model needs some refinement if it is to be applied to determine PMI of remains located in Greater Western Sydney.

8.4 Development of a new formula for PMI estimates in temperate Australia

It was found in the present study, the current methods and scoring protocols for PMI determinations do not accurately estimate time since death when applied to remains in this region. As a result, the Marhoff-Beard algorithms were created; one to be used if remains are believed to be deposited during winter and are located in a shaded microclimate, and a second winter formula to be used if the body is located in an open location in direct sunlight. The other two formulas are to be used if remains are postulated to have been deposited in summer; one for remains deposited in a shaded microclimate and one to be used when remains are in direct sunlight or an open location. The Marhoff-Beard method incorporates the most significant variables which were found to affect the process of soft tissue decomposition in a temperate Australian environment such as Greater Western Sydney. These variables were found to be humidity, rainfall and wind speed. To quantify decomposition, the body is divided into five regions. Each region is assigned a percentage, according to the 'Rule of Nines' (see Chapter 7), based on the surface area of that region as part of its contribution to the total body. By defining the surface area of each body region, it is believed this may reduce the ambiguity associated with making an overall full body assessment of the degree of decomposition.

The process for quantifying decomposition for its inclusion in the Marhoff-Beard formula may have contributed to the accuracy of this method. Difficulties have been found by previous researchers when using current scoring protocols, where they

report that the application of the scoring protocol was too ambiguous and open to bias, the protocols required the investigator to 'best estimate' the degree of decay that has occurred (i.e. the methods were too subjective) or they require the investigator to 'fit' the observed state of decay into a category [72]. While estimating the degree of decay will always attract some subjectivity, it is hoped that by defining key characteristics to include in the anthropologist's assessment of decomposition and by defining the contribution of body regions to the body as a whole, the new scoring criteria can be more readily and confidently applied. Further validation of the applicability of the new scoring criteria would be of benefit to test for inter-observer reliability and error.

The current study found that ambient temperature did not have as great an impact on the rate of decay as previously assumed and was determined to not be statistically significant. When the aforementioned variables were incorporated in this new formula, the Marhoff-Beard method produced more accurate determinations of PMI when validated on alternate porcine model subsets and human remains both retrospectively from photographs and on scene, when compared to the three current methods tested in the present study.

The three current protocols [1-3] for PMI estimations evaluated in this study, all included temperature as a variable in their formulas, suggesting that temperature was one of the most influential variables affecting decay rates at the time those studies were executed. As mentioned above, the temperature variable was not found to be a statistically significant variable in the current study. When the published methods were applied throughout the four trials they could not accurately estimate the ADD and PMI when temperatures were obtained from the site of the remains or from the local weather station. Given this was not found to be a significant variable, perhaps this contributed to the inaccuracies found when the published methods were applied, as they relied on temperature to generate an accurate estimate of PMI. The Marhoff-Beard formula does not incorporate this as a variable and, as such, may be the reason why the Marhoff-Beard method can more accurately, and in some instances precisely, predict the PMI to within days of the true PMI.

While the Marhoff-Beard formula for PMI determinations can accurately estimate the time since death during the fresh and early post-mortem period, limited accuracy was achieved in calculating the PMI once remains moved through the advanced

decomposition stages. We postulated the Marhoff-Beard method performed most accurately during the early decomposition stages as a body generally decomposes internally during this period [100]. Therefore, the variables influencing decomposition are relatively constant between bodies during this time. As decomposition progresses, it becomes more heavily reliant on the external environmental variables to drive the decomposition process and, in turn, the decay process loses its uniformity [100]. Due to this change in the decomposition pattern, it is possible there are further factors which impact on the decomposition process as remains progress through these later stages.

A multitude of studies have been performed worldwide to examine the decay process in an attempt to narrow PMI estimates [18, 22, 44, 49]. However, limited accuracy has been achieved thus far, especially when remains are in the advanced decomposition stages [3, 87]. This was found to be true for the new Marhoff-Beard winter and summer formulas created in the current study. Perhaps a simple regression formula, such as, those developed in the present investigation that includes only simple catalysts like climatic variables, are not appropriate for decomposed remains that have extensively dried, skeletonised or mummified. Collaborating with a variety of different disciplines using a variety of methods may be needed to estimate the PMI of a set of remains when the more advanced decomposition stages are observed. As postulated by Buekenhout et al. [145], it may be that a more comprehensive, multivariate study of decomposition is needed that includes a number of extrinsic or environmental features, such as scavenging, botany, soil chemistry, insect activity, in addition to the intrinsic factors influencing decay rates, such as the change in microbiology of a body, bone degradation, presence of disease or drugs at time of death, body size and composition. By examining the effect of a wider variety of variables within a microclimate, PMI estimates could be further narrowed during the later decomposition stages.

When the Marhoff-Beard formulas were validated on extensively decomposed remains, it was found that they generally underestimated the actual PMI. As with the current formulas which also produced erroneous PMI determinations during this period, this is problematic as police and forensic investigators use this calculation to begin their investigation, refine the list of individuals the remains belong to,

substantiate witness testimony, narrow the potential suspect pool and more [6]. Imprecise PMI determinations may lead the investigation in the wrong direction.

This Marhoff-Beard method would profit from further testing to establish at what time point in the estimated PMI generated by this method it begins to become inaccurate. Knowledge of when the method loses its precision would give anthropologist's a window to confidently apply this method in a practical forensic setting when the body is observed to be within the early post-mortem period and achieve an accurate estimation of the PMI. Variables affecting the sequence and rate of decay of decomposed bodies discovered in the Greater Western Sydney region that are in the advanced stages should be examined further, as identifying these variables may increase the Marhoff-Beard methods ability to predict the true PMI if these variables are known and can be accounted for in the formula.

Only fourteen pig carcasses and six human remains were available to test the new formulas and scoring protocol. This sample size for validating these new formulas is relatively small and the number of samples and sample types (human and pig) was distributed unevenly across the two seasons. More rigorous testing of the Marhoff-Beard summer and winter formulas and the new scoring protocol should continue to be undertaken in the Western Sydney region to better understand its accuracy. Although the sample size of the validation study was smaller than the developmental study and uneven across the seasons, the study provided valuable information on the use of these Marhoff-Beard formulas as it showed the areas of success and limitations of this method.

This Marhoff-Beard method was created using a porcine model. While estimating the time since death of decomposed pig remains would not likely be a common forensic case, it is difficult to conduct human studies in Australia due to the many legal and ethical restrictions [146]. Obtaining a large sample size of human bodies also has financial restrictions and donor opportunities are limited [117]. As such, for a study of this magnitude it was necessary to use a porcine model due to the biological and physiological similarities to humans [57, 94, 146, 147]. It was then necessary to validate the Marhoff-Beard method on a human model to determine its replicability and accuracy for use in real forensic investigations.

The Marhoff-Beard method was successfully applied to both pig carcasses and human remains during the validation study. It produced consistent results for the two

sample types across the range of 'discovery' dates available in the present study, particularly in the early stage where the PMI was estimated on average to within three days of the known PMI. This is a promising result not only for the use of the Marhoff-Beard method, but it confirms that the use of pig remains is appropriate as a proxy for human remains when undertaking decomposition research in temperate Australian environments. Keough et al. [119] propose that when applying current scoring protocols, such as that by Megyesi et al. [1], an amended scoring protocol needs to be applied when testing the method on pig remains. The Marhoff-Beard method developed in the current study does not require an alternate scoring protocol and can easily be applied to human and pig remains for research purposes.

The success of the Marhoff-Beard method is likely attributable to its specificity, in that it was created within a temperate Australian environment for use in PMI determinations of remains found in a temperate Australian environment. The variables included in the formula were all found to be the most influential climatic variables driving the decomposition process in the Western Sydney region. Cockle [121] stated that estimating the PMI of human remains based on the observed state of decay is 'impractical' and 'irresponsible' as decomposition is too variable. It has been shown in the present study that estimating the PMI by using the state of decay in conjunction with further variables can produce accurate PMI determinations.

It is acknowledged that there are limitations to using the Marhoff-Beard method, as there is when applying any method for PMI determinations. These limitations include its use once remains progress to the advanced decomposition stage and its use in other geographic regions as the method was created for specific use in a Western Sydney region. However, it is felt that a significant contribution to the knowledge of the decomposition process within this region has been made and the aims have been achieved where the purpose of this study was to narrow PMI estimates for the regions.

Chapter Nine

9 Conclusions

The results of the current investigation show that methods for PMI determinations need to be developed for individual geographic locations and climates. It is reported in the literature that geographic specific methods are of increasing necessity as decomposition is so variable and is dependent on the environmental features in any given region [9, 12, 15, 18]. Developing methods such as the Marhoff-Beard method, which are specific to individual regions will strengthen the integrity of anthropological methods for PMI determinations in court. Methods based on the morphological changes associated with decomposition are often considered subjective, however, the Marhoff-Beard developed method in this study is not solely based on the decomposition changes but it also incorporates the specific environmental variables influencing decay in the Western Sydney region. Further validation of the reliability of the Marhoff-Beard method could strengthen the justification for its use in PMI determinations, with the potential to use it as a standard forensic protocol accepted by the Australian legal system.

9.1 Limitations of the present study

Although the aims of this research were achieved, there were some limitations of this study. Firstly, one aim of this study was to add to the knowledge of the decomposition process of remains in a temperate Australian winter and summer climate. It has been previously stated that longitudinal human decomposition studies are difficult to conduct on a larger scale due to the ethical, financial and donor availability restrictions [146], but a suitable analogue for humans is the use of pig carcasses. While a porcine model was used as a proxy for human remains, it would be of benefit to study the decomposition process of human remains to gain a better understanding of the similarities and differences observed between these two sample types in this region. However, it is felt that the information reported is of substantial forensic value.

Secondly, the methods evaluated in the present study developed by Megyesi et al. [1] and Vass [3] were originally created from human remains. In the present study, their accuracy was validated using a porcine model. As these methods were designed to estimate the PMI of human remains, perhaps the use of a different sample type (i.e. pig carcasses) contributed to the flaws and inaccuracies found when applying these methods.

Thirdly, these methods, with the exception of the Megyesi et al. [1] ADD method, have not been widely tested both internationally and nationally. This makes it difficult to draw comparisons between other studies and other geographic regions and, therefore, makes it difficult to make definitive conclusions regarding the applicability of these methods for use in PMI determinations in Australia.

The Marhoff-Beard method created for PMI estimation in a temperate Australian environment is accurate up until the beginning of the advanced decomposition stage but loses its accuracy beyond this point when remains dry. It would be of value to the success of the Marhoff-Beard method to determine at exactly what time point the method becomes compromised and what variables during these later stages of decay are influencing the decomposition process, to then attempt to account for these variables in the method or propose alternate methods or techniques to estimate PMI in these later decomposition stages. It would also be of benefit to test the method when applied in the autumn and spring seasons to determine if its accuracy is continued, as only the winter and summer seasons were examined in the current study.

Furthermore, the pre-treatment of the human remains used in the study may have influenced the decomposition process. Prior to receiving the donated human bodies for this study, they were transported from their place of death and refrigerated until they could be placed at the research site. While cooler temperatures are said to delay the decomposition process [108], before they were refrigerated they may have begun decomposing in a different environment that is unlike Western Sydney and as such, could not be controlled for in the present study. The pre-treatment of pigs prior to slaughter may also affect the results. If the animal is stressed prior to death from either being transported to the abattoir and where it is held at the abattoir, this can affect the rate of decay. Studies have shown that any stress or exertion immediately prior to death that increases the blood pressure and heart rate can increase the onset

of decay [148]. Further testing should look at the ante-mortem variables immediately preceding death (e.g. presence of disease or medications) and the post-mortem variables (e.g. refrigeration for transport) which may affect decay rates, to determine if this will affect the validation of PMI methods.

Finally, only one driving aspect of decomposition was evaluated in the present study; climatic variables. To further understand the decomposition in this unique location, future studies should investigate the other factors affecting decomposition in this region. For example, it has been proposed that the eucalyptus oil from the eucalyptus trees may have a toxic effect on remains [149]. Eucalyptus trees are abundant in the Western Sydney region and future studies would profit from understanding the effects when a body is place under the canopy of these trees. Vertebrate scavenging was also not investigated in the present study. Red Foxes (*Vulpes vulpes*), Australian Ravens (*Corvus coronoides*), rats (*Rattus rattus*) and Wedge Tailed Eagles (*Aquila audax*) are all present in Australia and have been found in previous studies to be potential scavengers which can cause a devastating effect to the soft tissues and bones of a set of remains [150, 151]. It should be examined how scavengers affect the decay rates and the application of PMI methods.

9.2 Future work

To achieve more conclusive results regarding the accuracy of currently published methods for PMI determinations and their applicability in temperate Australian environments, further testing of the Megyesi et al. [1] Marhoff et al. [2] and Vass [3] formula should be undertaken, as they may be applicable in other Australian environments.

Only the summer and winter seasons were examined in the present study. Future research should examine each season within temperate Australia to evaluate their effect on the decomposition process of remains. The published methods for PMI determinations should also be tested during all seasons to determine if this influences the accuracy of the estimations.

Further taphonomic variables affecting the decay process within this region, such as soil type and composition, clothing and coverings, should be investigated. Specifically, future research should identify variables driving the decay process in

the later decomposition stages. This will help address the inaccuracies associated with time since death estimations during the more advanced decomposition stages.

Future research should expand on the present study and test the Marhoff-Beard formula in other regions of temperate Australia. Research should also investigate the variables influencing decomposition within their individual geographic regions and attempt to create novel methods for time since death estimations specific to their location. Validating the reliability of this novel method may potentially progress it to becoming an accepted standard method used in forensic investigations.

Chapter Ten

10 References

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Chapter Eleven

11 Glossary

Accumulated degree days: cumulative total of average daily temperatures over a period of time [152]

Adipocere: waxy or greasy substance formed during decomposition as a result of the hydrolysis and hydrogenation of tissue fat [153]

Autolysis: the self-digestion of cells via an enzymatic reaction takes place [154].

Cadaveric Island: the region below and surrounding a body where decomposition fluids have leeched [155]

Desiccation: the process of soft tissues dehydrating and drying, with the end result being mummification [87]

Lividity: a purple-red discolouration observed on the body where the blood has settled after the death of an individual as blood can no longer circulate throughout the body [156]

Mummification: the complete dehydration of a body. Mummification is a process which reduces the body to a structure light in weight and void of moisture [71]

Oviposition: to deposit or lay eggs by an insect [157]

Post-Mortem Interval: the time that has transpired from the time of death until the date the remains are discovered [8]

Putrefaction: destruction of the body's soft tissues by microorganisms [39]

Taphonomy: the discipline or study of the environmental conditions and processes which affect the preservation of an organism after death [158]