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THE INFLUENCE OF REHYDRATION ON DECOMPOSITION IN THE HIGHVELD REGION OF SOUTH AFRICA, USING A PIG MODEL

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A dissertation submitted to the Wits School of Anatomical Sciences,

Faculty of Health Science, University of the Witwatersrand in

fulfilment of the requirements for the degree of Master of (Med) Science in Anatomical Sciences

Johannesburg

2022

Declaration

I declare that this dissertation is my own unaided work. It is being submitted for the degree of Master of (Med) Science in Anatomical Sciences at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.



Claire Lynne du Toit

23rd day of June in the year 2022

Presentations arising from this research project

Poster presentation given at the Anatomical Society of Southern Africa conference hosted virtually by the University of the Western Cape in April 19-22, 2022

Abstract

Rainfall has been reported to influence decomposition with the timing of the precipitation, causing either an increase or decrease in decomposition rates. Researchers have observed that carcasses exposed to rainfall may re-initiate decomposition in desiccated tissue. However, no conclusive research-based evidence exists on the specific effects of rehydration on decomposition. Therefore, this study aimed to assess the effects of artificial rehydration on the rate and progression of decomposition after remains have stalled in the advanced stage of decomposition. Twelve adult pig carcasses (40-100kg) were set out in the central Highveld of South Africa during cooler (April – July 2021), and warmer (July – November 2021) climates, and decomposition was scored ±three times a week to obtain the total body score. All carcasses (8 = experimental; 4 = controls) were covered by chicken wire cages with transparent tarps to control for external factors and prevent natural rehydration. Once the experimental carcasses reached a three-visit stasis in the advanced phase of decomposition, they were artificially rehydrated, and decomposition was further observed. The trials were split into cooler and warmer deployments, and analysis of the data indicated changes in the progression and rate of decomposition between the control and experimental groups. The rehydrated experimental carcasses showed re-initiation of decay, along with insect re-colonization, while the control carcasses mainly remained in a state of stasis and insect activity ceased altogether. It was also noted that the experimental group displayed greater cadaver decomposition islands. Results indicate that both direct and indirect rehydration increases the rate of decomposition and also changes the progression thereof. Future research to further understand how rehydration (artificial and natural) impacts decomposition progression during its various stages along with invertebrate colonisation, will enhance our understanding of effects of these environmental factors have on the accuracy of post-mortem interval estimation.

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List of Abbreviations

ADD	Accumulative Degree Day
ATP	Adenosine Triphosphate
CDI	Cadaver Decomposition Island
CP	Control Pig
Cwb	(C) Temperate, (w) dry winter, (b) warm summer (Köppen climate)
EP	Experimental pig
FABF	Forensic Anthropology Body Farm
ICC	Intraclass correlation
PMI	Post-mortem Interval
TBS	Total Body Score
TDS	Total Decomposition Score
TSD	Time since death
VOC	Volatile Organic Compound

Chapter 1 Introduction

A major focus in forensic taphonomy is the process of decomposition and all the biotic and abiotic elements that play an active role in its progression or cessation. Decomposition is considered a systematic breakdown of a whole body until the point of skeletonisation (Galloway et al., 1989). The process of decomposition consists of various stages, and although these stages of decomposition have been delineated, there is significant overlap (Tabor et al., 2004). Knowledge of the decomposition process is vital when assessing the post-mortem interval, especially understanding how the various factors influence the rate of decay during the different stages of decomposition.

The time that passes between the discovery of remains and death is known as the post-mortem interval (PMI). The PMI is a critical tool utilised in death investigations that can provide context to the recovered remains. For example, PMI can allude to post-mortem environmental conditions a body may have been exposed to and may aid in the identification of the individual by narrowing down the list of possible missing persons (Megyesi et al., 2005). In South Africa, the number of unidentified remains is high. For example, of the 18 324 bodies stored at 11 Medico-legal Laboratories across Gauteng in 2020, approximately 1 173 (6.4%) remained unidentified (Bloom, 2021). However, this high number of cases involving unidentified decomposed individuals and skeletal remains is not a local, but also a global issue (da Silva et al., 2009), highlighting the need for better means of identification, including more accurate methods to estimate the PMI.

Current methods used to estimate the PMI are linked to the visible progression of decay of human remains (Clark et al., 1997; Galloway, 1997; Megyesi et al., 2005). Megyesi and colleagues (2005) developed the most frequently used method to quantify the decomposition process, via PMI estimation that focuses on the progression and subsequent "stage" of decomposition the remains have reached. This method requires quantifying the point of decomposition of the remains and then correlating this value to the amount of thermal energy required for remains to reach this stage of decomposition.

Some of the main factors influencing decomposition rates include temperature, humidity, rainfall, soil, invertebrate colonisation, vertebrate scavenging, trauma, clothing, as well as, body size and weight. Of these, temperature, humidity and rainfall are the most influential (Mann et al., 1990). Rainfall has been attributed to having a great effect on the rate of

decomposition, but there is limited research looking at the direct effects of rainfall and rehydration on the rate and progression of decomposition.

Although little is known about the effects of rehydration on decomposition, it has been suggested that it will result in the re-initiation of active decomposition once decomposition has plateaued in the advanced stage (Myburgh et al., 2013). The effects of rain (rehydration) have mainly been observed as a part of seasonal variation (Archer, 2004). This is forensically significant, as remains are likely to dry out during dry seasons, yet likely resume decomposition after rehydration (rainfall) in the wet season. If these remains are therefore only discovered in the wet season, a shorter PMI estimation than the actual PMI will be calculated, therefore providing inaccurate results. This is specifically the case in the Highveld region of northern South Africa with its colder and drier autumn and winter months (April - September).

1.1 Study aims and objectives:

This study aimed to address the lack of evidence regarding the influence of rehydration on remains after having entered a state of stasis in the advanced stage of decomposition. To accomplish this aim, an experimental group of pig carcasses were artificially rehydrated after a period of decomposition. Artificial rehydration served as a means of controlling the timing and quantity of water the remains were exposed to. The rates of decomposition were compared to a control group of pig carcasses. The following objectives were set to achieve the aims of the study:

1. To establish a method to allow for the artificial rehydration of pig carcasses in a field setting. This will be accomplished by testing out a cage design and tarp covering for each sample pig to protect the sample from direct natural rehydration and allow for artificial rehydration with a watering can.
2. To investigate the effects that the rehydration of pig carcasses has on the decomposition rate and progression thereof. This will be evaluated by collecting and observing the total body score of rehydrated (experimental sample) versus non-rehydrated carcasses (control sample) and comparing the progression and rate of decomposition over time.
3. To observe the effects that rehydration of the pig carcasses has on invertebrate scavenger activity, through observation of presence or absence on the carcasses pre- and post-rehydration.

After the carcasses have stalled in advanced decomposition, it was hypothesised that the introduction of water (artificial rehydration) will rehydrate the desiccated tissue, re-introduce a new wave of invertebrate colonisers and, therefore, re-activate decomposition after a period of inactivity.

Chapter 2 Literature Review

Taphonomy studies investigate the processes that affect animal and plant remains as they transition "from the biosphere to the lithosphere" and become fossilised (Efremov, 1940). The progression of decomposition is a fundamental process in forensic taphonomy. This is because the way remains decay over time can be used to estimate the time since death, or PMI, in a forensic context. This study's focus is the process of decomposition, which is affected by numerous factors, including temperature, humidity, and rainfall. This review will explore the current literature, providing background and insight into the present state of research in the field of forensic taphonomy as it pertains to the study of PMI estimation and the role that intrinsic and extrinsic factors play on the rate of decomposition.

2.1 Decomposition

Many research studies have observed the well-defined processes of decomposition (Vass et al., 1992; Clark et al., 1997; Keough et al., 2017; Finaughty & Morris, 2019). It is explored in this section to understand the process and comprehend how different environmental factors play a role in its progression and cessation.

Decomposition consists of two major processes; autolysis and putrefaction. Upon death, the process of autolysis initiates soft-tissue decomposition progression (Clark et al., 1997). Autolysis is the process where intracellular fluids and nutrients are released into the surrounding tissues and become the primary food source for internal bacteria (Clark et al., 1997). The microscopic process of autolysis begins once breathing ceases and the body becomes oxygen-deprived. Cellular oxygen deprivation increases carbon dioxide concentration, which decreases the cellular pH (Vass, 2001). The accumulated waste in the cell then triggers a poisonous reaction dissolving the cell from the inside out, causing it to rupture. Gross anatomical changes are usually only visible a few hours after death. The first observable changes include fluid-filled blisters and skin slippage. Other changes associated with autolysis include algor mortis (body cooling to ambient temperature), livor mortis (discolouration due to blood pooling), and rigor mortis (body stiffening) (Vass, 2001). Once enough cells have ruptured, the released nutrients are the catalyst for the following major decomposition process: putrefaction.

Putrefaction or anaerobic decomposition is the process of degradation and decomposition of organic matter, with rapid loss of biomass, eventually resulting in skeletonisation (Clark et al.,

1997). This process results in dramatic soft tissue changes. This catabolism of tissue into gases, liquids, and simple molecules are first observed in the settling of Sulfhaemoglobin in the blood, displayed as a green discolouration in the abdomen. The nutrients released from the cell start the proliferation of the micro-organisms (bacteria, fungi, and protozoa populations) that begin digesting the internal tissues (Clark et al., 1997). The metabolic by-product of the bacterial tissue consumption is gas production, which leads to bloating and eventual purging of fluids and gases from the remains' orifices. During this process, the semiochemicals (cadaveric volatile organic compounds) attract vertebrate and invertebrate scavengers to the remains, including flies, beetles, mammalian species, and avian scavengers (Pascual et al., 2017).

Decomposition is considered a continuous process with overlapping physical, chemical, and biological changes (Tabor et al., 2004). However, when developing PMI estimation methods, it has been observed to be phase-specific. It is, therefore, advantageous to divide decomposition into specific stages as this facilitates the measurement, organisation, employing methods, and discussion of scientific research (Schoenly et al., 2016). Typically, the decomposition process has a specific sequence starting at the fresh stage, followed by the bloat, putrefaction, and finally ending in the skeletonisation stage (Galloway et al., 1989; Vass et al., 1992). The stages of decomposition have been defined many times, varying from two to nine stages (Vass et al., 1992; Goff, 2009). It is, however, common in forensic taphonomy to define decomposition stages as fresh, early decomposition, advanced decomposition, and skeletonisation (Megyesi et al., 2005; Keough et al., 2017).

Some environmental variables will accelerate the rate of decay, while others will decrease the rate or, in specific cases, result in the complete cessation of decomposition, and subsequent preservation (Campobasso et al., 2001). For example, adipocere formation, also known as "Grave wax, " is a preservation process that occurs in a moist, oxygen-deficient environment. The remains' body fat is saponified (hydrolysis of triglycerides into glycerine and fatty acids) (Forbes et al., 2002) and turned into a wax-like material that can remain stable for many years preserving injuries that can help inform about the cause of death (Gupta & Jain, 2011). Mummification is another form of preservation where the body's soft tissues dry out, caused by an extreme lack of moisture. These forms of preservation are brought on by environmental conditions that induce preservation as opposed to complete degradation.

Gross anatomical changes are used as indicators of PMI and what taphonomic and environmental forces have acted on the remains. The information gathered about the stage of

decomposition and the subsequent PMI will help answer one of the main questions in forensic death investigations; *when* did the victim die? (Vass, 2001). Methods of PMI have been a focus of forensic sciences and have been highly linked to decay progression. However, this progression is influenced dramatically by the environment (Mann et al., 1990).

2.2 Post-mortem interval

The PMI is the time that has elapsed between death and the recovery of remains. In a forensic setting, having a timeline of events following death allows for narrowing down the victim/s that could be involved. The PMI can also provide a possible corroboration of witness testimony and narrow the list of suspects of interest in the death investigation (Megyesi et al., 2005). Considerable focus is placed on the development of PMI estimation methods in decomposition research. Recently, there has been a pivotal shift towards more quantitative PMI estimation methods; this has been a move across forensic disciplines (Dirkmaat et al., 2008). Precedent-setting court cases triggered a change in research focus by outlining what is considered valid science/evidence admissible in court, leading to the Daubert standards (Christensen & Crowder, 2009). The move led Henssge and Madea (2007) to propose four criteria for PMI estimation methods to gain practical relevance: (1) quantitative measurement, (2) mathematical description, (3) considering influencing factors quantitatively, and (4) proof of precision on independent materials. The difficulty in standardisation comes from the many factors that play a role in the progression and cessation of decomposition and the move towards quantifying methodology. The following is an analysis/breakdown of the current techniques used for PMI estimation in forensic sciences.

2.2.1 Post-mortem interval estimation methods

Methods of PMI estimation are used from shortly after death to skeletonisation. The post-mortem timeline has traditionally been categorised into three stages – immediate, early, and late (Chavali et al., 2008). There have been methods devised to estimate PMI within these time frames.

In the immediate stage of post-mortem, the body goes through rapid biochemical and physiochemical changes. These changes are brought on by the cessation of blood circulation through the system. The changes observed during the immediate phase are visible on the skin and the eyes (Chavali et al., 2008). The clouding of the cornea can be present within two hours after death. A loss of intraocular pressure takes place, which can indicate PMI for up to six

hours. Skin loses elasticity within hours after death (2-3 hours PMI), but histological analysis shows no morphological changes up to six hours post-mortem (Bardale et al., 2012).

Early PMI is associated with the onset and potential measurement of rigor, livor, and algor mortis occurring from 3 - 72 hours after death (Mathur & Agrawal, 2011). The early PMI phase assessment is crucial, as most medico-legal cases are examined at this period (Chavali et al., 2008). These methods, although beneficial, are greatly affected by the specific environments and inter-individual variability of the victim (Knight, 1997; Tracqui, 2000). Rigor mortis is a PMI estimation method that quantifies a time frame of post-mortem muscle stiffening caused by depletion of adenosine triphosphate (ATP) in the muscle tissue. ATP breaks down the actin-myosin filaments in muscle fibres; the two components form covalent bonds within the fibres. The cessation of oxygen to the body results in the lack of ATP production and stiffening of the muscles (Chavali et al., 2008). The onset of rigor mortis appears approximately 2 hours after death and remains until 24 hours after death (Anders et al., 2013). Rigor mortis ceases when the actin-myosin complex denatures due to proteolysis, resulting in stiffness termination and generally rigor disappears 36 hours after death (Chavali et al., 2008).

Algor mortis is used as a PMI measure concerning the loss of body heat equalising to ambient temperature. This method of PMI estimation is most accurate during the first 24 hours after death. The accuracy of this method depends on various factors, such as different temperatures within the same localities. A method developed by Henssge and Madea (1988) made use of the rectal temperature time of death nomogram, utilising formulae, several charts and algorithms and is the most widely taught. However, Bartgis et al. (2016) conclude that a "one-size-fits-all approach" would lead to the incorrect estimation of PMI and proposed a database of cooling charts considering important factors, such as body size, shape, and environmental conditions. Livor mortis is the last change within the triad and is described as the pooling of blood in skin vessels caused by gravitational pull. The initial onset of discolouration induced by livor mortis starts to appear approximately 3-4 hours post-mortem and is fixed 6-12 hours post-mortem (Tsokos, 2005).

PMI estimation using livor mortis can be measured using spectrophotometric analysis or confirmation of fixation by pressure-induced blanching (Kaatsch et al., 1994). This method is only valid until 12 hours after death as the lividity becomes fixed after this time frame. Lividity analysis methods developed by Usumoto et al. (2010) managed to estimate PMI within ±4.76 hours. Other methods considered within the early PMI phase look at the biochemical markers

in blood and body fluids that break down as the body progressively decays (Donaldson & Lamont, 2014). The pH of blood serves as a biomarker, seen to increase in acidity 20 hours post-mortem (Hachem & Sharma, 2019). Post-mortem lactase is also a marker in the blood increasing its concentration 20 times in one-hour post-mortem and 70 times in twenty-four hours post-mortem.

Late PMI estimation is based on the rate and progression of body tissue disintegrating. The progression of decomposition is used as the timeline for PMI. As explained above, the stages of decomposition (fresh, early, advanced, and skeletonisation) are linked with specific characteristics. These are used to estimate where the remains are in the decomposition cycle; this is then correlated to a timeline. The fresh stage takes place 1-2 days post-mortem where the body's cells are deprived of oxygen. The early decomposition stage is characterised by the start of bloating and the initiation of putrefaction. The early decompositional stage can begin from the first post-mortem day up to five days post-mortem seen with maggots appearing on the remains, discolouration of skin and bloating (seen as early as three days post-mortem). The advanced decomposition stage is associated with the remains having a blackened appearance, the collapse of the abdomen, sagging of skin, and extensive maggot colonisation and less than half of the skeletal material being exposed. The advanced stage of decomposition takes place approximately 11-24 days post-mortem. The skeletonisation stage is associated with more than half of the skeletal material being exposed from 24 days onward. The timeline associated with these stages is highly dependent on the environmental factors which influence their progression (Mann et al., 1990).

The current lack in consistent and reliable quantitative methods of PMI estimation have resulted in researchers directing efforts into quantifiable models of PMI estimation. There are PMI estimation methods being developed to analyse "aroma" from the body (Xia et al., 2019). The volatile organic compounds (VOCs) produced from rat muscle is being measured at different points of PMI, and this is being proposed as a new method of PMI estimation from two to ten days post-mortem (Xia et al., 2019). This research is a starting point to test methods on human muscle tissue. Investigation into "thanatochemistry" (chemical changes on and in the body) focusing on the decrease in microbial diversity as a measure of PMI with efficacy shown after 12 hours post-mortem (Martínez Aragonés et al., 2022; Zapico & Adserias-Garriga, 2022). An entomological study conducted by Mok et al. (2021), explores the presence and quantification of proteomics and metabolomics in maggots that have been extracted off

human remains for PMI estimation. Multiple researchers have been looking into dental analysis focusing on cellular degeneration, including RNA and dental pulp (Mehendiratta et al., 2015; Carrasco et al., 2017). The decay of dental pulp within human teeth is also being assessed as another PMI estimation (Bhuyan et al., 2020). Bhuyan and colleagues (2020) attempted to observe morphological and microscopic features of dental pulp over time. This method proves useful up to two years post-mortem, but it is a qualitative study describing changes in colour as "pink at 24h and pale pink at 72h" and is hard to quantify. PMI is better estimated by incorporating both qualitative and quantitative methods. This is due to the factors involved in the decomposition process being highly variable and beyond the examiners' control in real-life situations (Mathur & Agrawal, 2011).

2.2.2 Accuracy and testing of post-mortem estimation methods

Megyesi et al. (2005) constructed a temperature-based model of PMI estimation. The research made use of 68 human remain cases with known dates of death. Megyesi and colleagues (2005) developed a model to estimate PMI using thermal energy, measured in accumulated degree days (ADD), and a quantitative (semi-continuous variable) measure of decay, known as total body score (TBS). This method focuses on the sum of thermal energy required to progress decomposition to a certain point. Decomposition is visually assessed and then assigned a TBS correlated to the degree of decay. The TBS is divided into three separate regions of the body. The separate scoring of these categories is due to rates of decomposition differing between the regions; head and neck, trunk, and limbs. The varying scores are associated with the differential compositions of each of these regions and differing rates of decay i.e., the limbs do not bloat as severely as the trunk and insect colonise the head and anus first, leaving the limbs until later in the decay process (Megyesi et al., 2005). The scores for the head and neck range from 1 to 13, trunk 1 to 12, and limbs 1 to 10, representing the fresh stage to the skeletonisation stage. The sum of each score allocated to the separate regions of the body produce the TBS (Megyesi et al., 2005). Once the TBS has been calculated, a regression formula is used to calculate the ADD. The ADD is then converted to an estimated number of days since death based on the local region's average daily temperatures. Gelderman's total decomposition score (TDS) proposed a similar method, also making use of ADD. The TDS method separates the head and neck into different categories and is calculated as the sum of the facial, body and limb decomposition scores. The separation of the head and neck was justified from observations of

differing rates of decomposition. These scores range from one being fresh and six being skeletonised (Gelderman et al., 2018).

Researchers and law enforcement commonly used this TBS/ADD method proposed by Megyesi and colleagues (2005) (Simmons et al., 2010; Myburgh et al., 2013; Sutherland et al., 2013; Card et al., 2015; Cockle & Bell, 2015; Jeong et al., 2016; Vidoli et al., 2019), however, when validating the study in various countries, the method did not show accurate results. Moffatt et al. (2016) identified errors in the Megyesi et al. (2005) predictive formula. The errors identified included concerns about the statistical treatment of the data, rounding errors, inappropriate temperature scales and inaccuracies of entomological derived PMI estimations used as the precise time of death (Moffatt et al., 2016). The research team proposed a new formula and a redefined scale for TBS to range from 0 to 32 (Moffatt et al., 2016). Regional validation for the Cape Town, South African climate was conducted by Forbes and colleagues (2019) for both the Megyesi et al. (ADD_{Meg}) method and the Moffatt et al. (ADD_{Mof}) method. The researchers found that when estimated values rendered from these formulae were compared to actual ADD values, the estimated ADD was inaccurate for both models. The estimated ADD were compared to the actual ADD values and the two models proved inaccurate during winter, however the models were partially accurate during the summer months. The Moffatt et al. (2016) model demonstrated greater accuracy in the early stages of decomposition, and Megyesi et al. (2005) model in the later stages of decomposition (Forbes et al., 2019). Another validation study was conducted in the Highveld region, Gauteng, South Africa by Myburgh et al. (2013), whereby pig carcasses were used to assess the accuracy of using ADD for PMI estimation. The method did not prove accurate, and upon validation only one out of 16 pig carcasses fell within the 95% prediction interval (Myburgh et al., 2013). These findings support the need for regional and seasonal specific formulas for estimating PMI in a forensic context or developing complex models incorporating "big data" to evaluate all the different variables that influence the rate and progression of decay (Forbes et al., 2019). A study conducted by Wescott et al. (2018) attempted to validate Megyesi's TBS method utilizing human remains with known ADD and PMI, proving that these were not very accurate and highlighted the effect of variable environments, i.e., shaded and sunny environments displaying different insect activity. A more current research study put forward an improved model based on Megyesi et al. (2005)'s method. The method involved scoring 16 regions of the body independently to minimise scoring bias (Vidoli et al., 2019). Vidoli et al. (2019) included scavenger activity and hot spot analysis to address more variables affecting the rate of decay.

2.3 Factors affecting decomposition

To understand the progression of decomposition concerning time, it is essential to grasp how different drivers affect the rate of decay. Variation in the taphonomic processes was defined into three categories by Nawrocki (1995): 1) Individual factors – which are intrinsic factors linked to variables concerning the remains, e.g., body size, trauma, and disease (Campobasso et al., 2001; Cross & Simmons, 2010; Zhou & Byard, 2011; Hayman & Oxenham, 2016); 2) Behavioural or cultural factors – caused by human activity involving the location of the body namely burial depth, soil composition, submersion and body covering (Dent et al., 2004; Zhou & Byard, 2011; Spies et al., 2020); and lastly 3) Environmental factors – external variables are subdivided into biotic (living) factors such as invertebrate and vertebrate scavenging and influence of flora, and abiotic (non-living) factors such as temperature, sun exposure and rainfall. The effects of these factors on the rate of decomposition on human remains are discussed below.

2.3.1 Individual factors

Individual factors, also known as intrinsic variables, are those factors that are present within the carcass/remains ante-mortem (existing and active before death) or perimortem (existing and active around the time of death). Ante-mortem intrinsic variables include body size and post-mortem intrinsic variables being trauma, health status or disease (Nawrocki, 1995).

Body Size

Kneidel stated that greater carcass sizes resulted in greater biomass and surface area to volume ratio. Hence, decomposition progression and rate would differ compared to a smaller carcass in any given environment (Kneidal, 1984). Research conducted by Hewadikaram and Goff (1991) concluded no significant difference in the rate of decay should the carcass weight differ by ±10kg. Researchers then started using carcasses of the same size during research to control for this factor. However, a trend of using carrion weighing 23kg was soon contested as it incorrectly mimicked a forensic situation and hindered the use of the data for scientific inference (Catts, 1992; Goff, 1993). More recent research conducted by Sutherland et al. (2013) highlighted that smaller bodies (< 35 kg) decomposed four times faster than larger bodies (60 – 90kg). When looking at body size, decomposition rates also depend on body composition. A larger body has more tissue to decompose. In some cases, a larger body can be leaner than a smaller body and decompose faster. The larger body will appear to have a slower rate of decay

due to the amount of tissue that needs to be decomposed (Sutherland et al., 2013). Higher fat content in a body, results in faster decomposition due to liquefaction of the body fat (Mann et al., 1990). The subcutaneous fat present in an obese individual has an insulating effect, subsequently slowing the rate of cooling of the body. This highlights the individual factors that affect the rate of decomposition, not only due to body size but also composition.

Trauma

Trauma has been considered among the factors that affect the rate of decomposition (Micozzi, 1986; Vass et al., 1992; Campobasso et al., 2001). Trauma is defined as serious injury to the body, for example, deep cuts, broken bones, or open wounds. These traumatic incidences may or may not be linked to the cause of death. Initial studies proposing that trauma to remains will increase the rate of decay, when compared to the normal pattern of decomposition, includes the studies published by Micozzi (1986) and Mann et al. (1990). Micozzi (1986) based his conclusion on the effects of trauma on the rate of decomposition using previously frozen rats with their cervical vertebrate dislocated. The finding in this research should however be interpreted with caution as freezing affects how remains decay (Micozzi, 1997). The study focused on the effects of internal trauma and researches should be cautioned when applying it to external trauma and using the findings as a generalisation. Mann et al. (1990) observed the effects of trauma in a comparative study between a person who has sustained a perimortem gunshot wound to the thorax and a person without any noticeable trauma. Mann et al. (1990) noted that the gunshot victim decomposed faster than the individual without trauma. However, the researchers provided no quantitative or qualitative data (Mann et al., 1990), and caution should be observed due to the small sample size and the possible role that individual variation plays in the rate of decomposition. More recently, researchers have found evidence to the contrary (Kelly, 2006; Kelly et al., 2008, 2011; Cross & Simmons, 2010).

Research conducted by Kelly et al. (2011) found that pig carcasses with trauma did not decompose at an accelerated rate. Insects are one of the most influential variables affecting the rate of decomposition (Mann et al., 1990). It was hypothesised that the insects would use the trauma sites as an oviposition site, however Diptera did not favour these sites (Kelly et al., 2011), but rather the natural orifices (Cross & Simmons, 2010). Smith (2014) studied the effects of sharp-force trauma and found that the decomposition pattern may be altered by trauma but not the decomposition rate. The type of trauma (stab, slashed, and no wound) did prove to differ in the pattern and rate of decay within the first twelve hours, being greater for

the stab wounds when compared to slashed wounds and no wounds inflicted on the pig carcasses (Munro et al., 2019). The assertion that trauma is a significant factor considered in the rate of decomposition (Mann et al., 1990), is currently being challenged (Smith, 2014; Munro et al., 2019; Miles et al., 2020). More research on a human sample is required to simulate a more realistic scenario regarding trauma with a forensic application.

Disease

As decomposition is initiated, autolysis and putrefaction start the breakdown of the remains (Goff, 2009). When remains have endogenous factors playing a role in the death or condition the body is found in, such as disease and subsequent body temperature, it can affect the rate of decay (Zhou & Byard, 2011). After death, the production of heat from the body ceases. The bacteria present inside remains use the body as a medium for growth, and needs the warmth of the body for proliferation. The most important factor in determining the rate of decomposition, is the temperature of the body and the environment post-mortem. An increased body temperature at death can be attributed to heat stroke, fever, intoxication of illicit drugs, malignant hyperthermia, and hyperthyroidism (Byard et al., 1998; Green et al., 2001; Tsokos, 2005). Reduced body temperature can be attributed to hypothermia, congestive heart failure, severe fatigue, stroke, or Parkinson's disease (Micozzi, 1997; Linares et al., 2016; Villanueva-García et al., 2021). Infectious diseases also increase the rate of decomposition due to increasing ante-mortem bacterial load and the probability of increased body temperature at the time of death (Campobasso et al., 2001; Tsokos, 2005). Literature indicated that an individual's health status needs to be considered when interpreting PMI estimations as misinterpretation can confuse witness testimony and slow identification efforts (Zhou & Byard, 2011).

2.3.2 Behavioural and cultural factors

The location of the body, how it is deposited, and preserved after death are considered extrinsic cultural variables, which all affect the manner and rate of decomposition. In a forensic context, how remains are deposited post-mortem greatly affects the rate of decomposition. These variables include the burial depth, possible submersion, and covering of remains (Rodriguez & Bass, 1985; Rodriguez, 1997; Kelly et al., 2009; Simmons & Heaton, 2009). Delineation between clothed, unclothed, surface, buried, and submerged decomposition is needed to understand the critical role they individually play in decomposition rate. Insects play a significant role in the rate of decomposition. If insects are hindered from access to remains,

there is a notable change in the progression and the rate of decomposition (Rodriguez & Bass, 1985). Not only are the remains limited in their insect exposure, but they are also shielded from the effects of solar radiation.

Burial death

Burial depth is a behavioural or cultural practice that plays a role in the rate of decomposition. Rodriguez and Bass (1985) studied the effects of burial depth on the rate of decay, laying cadavers into unlined trenches at different depths of 30 cm, 60 cm, and 1.2 m. The remains buried at the shallower depths decomposed at a faster rate than the remains buried at 1.2 m. The attributing factors were isolated to two main influences - restriction of insect activity due to soil surrounding the remains and soil acting as barriers to climatic factors.

Insects were observed burrowing to the cadavers in the shallow graves through the crevices in the soil made by bloating of the remains. The remains in the shallower graves give off VOCs that attract both vertebrate and invertebrate scavengers (Rodriguez & Bass, 1985). It was observed that the scavengers become alerted to the presence of buried remains and proceed to uncover and feed on them. A study conducted by Marais-Werner et al. (2018) comparing surface and buried remains, found the progression of decomposition to be similar, but the rates differed considerably. Remains found buried are subject to less insect colonisation, lack of access by vertebrate scavengers, and reduced temperature fluctuations (Rodriguez & Bass, 1985; Simmons et al., 2010; Marais-Werner et al., 2018). As the remains are buried deeper, the moisture and organisms in the soil change (Bachmann & Simmons, 2010). Therefore, buried remains are seen to decompose at a slower rate than surface remains and the decomposition rate decreases further the deeper the remains are buried (Mann et al., 1990; Kelly et al., 2009; Bachmann & Simmons, 2010; Marais-Werner et al., 2018). Plants located near the remains are attracted to the nutrient-dense decomposition fluid excreted by the remains and aid in the breakdown of the body, clothing, and skeletal material (Rodriguez, 1997). These root systems, insects, and microbial organisms are found closer to the surface and aid in the increased rate of decomposition in shallower clandestine graves (Rodriguez, 1997).

Soil composition also plays a role in decomposition rate as different soils contain different levels of moisture. For example, clay soil (soil with high moisture content) often results in delayed decomposition due to adipocere formation (Rodriguez, 1997). Researchers suggest that the decomposition enzymes become bound to the soil, rendering them inactive, resulting in a

slower rate of decay (Skujins, 1967; Turner & Wiltshire, 1999). A study conducted by Tumer et al. (2013), looking at the effects of different soil types on the rate of decomposition observed an increased rate for loam and organic soil when compared to clay and sandy soil. The findings were attributed to the composition of the different soils and their textures. Soils that contain more chemical and biochemical factors e.g., $CaCO_3$ and CO_2, which provide a nutrient source for greater microbial activity, resulting in an increased rate of decomposition (Tumer et al., 2013).

Submersion

A body that is submerged in an aquatic environment often follows a specific order of decompositional processes. The sequence of these events is similar to a body found on the surface but elicit a chain reaction of events specific to submerged remains (Simmons & Heaton, 2009). The body initially sinks as the air is expelled fully from the lungs. As the remains start the active phase of decomposition, with bloating in the gastrointestinal tract, the remains begin to resurface. Once the process of putrefaction starts with the purging of gases, the remains sink again (Boyle et al., 1997; Rodriguez, 1997; Dalal et al., 2020). The decomposition of aquatic submerged remains is noted to occur in approximately double the time of remains decomposing on the surface and exposed to air (Rodriguez, 1997). The slowed rate of submerged remains is attributed to the lower temperature and delayed insect activity resulting in preservation of tissue and the occurrence of adipocere formation, which halts bacterial growth (Rodriguez, 1997; Forbes et al., 2002; Simmons & Heaton, 2009; Dalal et al., 2020).

Methods of PMI estimation in aquatic situations have been redefined as post-mortem submerged interval (Dalal et al., 2020). Assessment of the aspects affecting the rate of decomposition of submerged remains include factors such as, temperature, aquatic scavengers, bacterial content, and salinity (Boyle et al., 1997; Rodriguez, 1997; Dalal et al., 2020). As with surface decomposition, temperature plays a major role in aquatic decomposition. The effect of temperature can be seen with remains in warmer waters resurfacing at a more rapid rate due to enhanced bacterial growth in the gastrointestinal tract, accelerating the process of bloating (Boyle et al., 1997; Rodriguez, 1997; Megyesi et al., 2005). There are greater occurrences of adipocere formation in submerged bodies, especially in warmer water, which inhibits decomposition and ultimately the investigator's ability to estimate PMI (Simmons & Heaton, 2009). The carcasses rate of decomposition in a submerged environment are highly linked to the access scavengers have to the carcass, as mentioned it has been observed that the initial

submersion of remains delays the access of insect activity to the body (Dalal et al., 2020). As the body floats to the surface, it is colonised by many terrestrial insects and aquatic insects, this access to the carcass progresses the process of decay similar to that of "land" decomposition (Dalal et al., 2020). The salinity of the water has a great effect on the rate and progression of decomposition when compared to remains found in freshwater. Remains located in saltwater will decompose slower due to reduced bacterial activity halted by the high salt concentration (Boyle et al., 1997; Rodriguez, 1997). Bacterial growth is promoted in freshwater, therefore, resulting in a greater rate of decomposition (Rodriguez, 1997).

Body covering

Body covering can be defined in the context of forensic taphonomy as the state a body is found in, being dressed/undressed, with blankets or other means of concealment. It is more common during a death investigation for a body to be recovered with a form of clothing or covering present (Spies et al., 2020). The effect of clothing on the rate of decomposition remains undecided, with research showing both accelerated and slowed rates of decomposition (Cahoon, 1992; Miller, 2002; Card et al., 2015). The presence of clothing created other factors to be considered, including the trapping of moisture, increased temperature, decreased access to vertebrate scavenging, new areas of oviposition for insects as well as protection from the elements (Cahoon, 1992; Campobasso et al., 2001; Miller, 2002; Card et al., 2015). In a multi-seasonal study conducted by Kelly et al. (2008, 2009), clothed and wrapped pig carcasses were studied to assess their effects on decomposition and arthropod succession. In both seasonal studies, the clothed and wrapped carcasses retained moisture for longer. A recent study assessing the effects of clothing on the rate of decomposition indicated that clothing served as a barrier for vertebrate scavengers resulting in a slower weight loss and a decreased rate of decay (Spies et al., 2020). However, the authors emphasised the importance of regional data as vertebrate scavengers differ in pattern and activity; for example, bears were not hindered by the presence of clothing (Carson et al., 2000). Therefore, it is important to understand how environmental factors play a role in the rate of decomposition. When regional studies are conducted, the elements should be fully understood to help with the interpretations of the state of decay.

2.3.3 Environmental factors

Extrinsic environmental variables are never independent of one another (Nawrocki, 1995). When assessing the effects environmental factors play on the rate of decomposition, interpretations of individual variables are done with caution and considering many influencing factors such as scavenger activity, temperature, humidity/aridity, and rainfall (Mann et al., 1990). Although each factor will be considered separately below, the effects of the factors will be explained with their consequential effects or influences on other environmental variables.

Invertebrate scavengers

Invertebrate scavenging is one of the most important factors affecting the rate of decomposition since invertebrate scavenging and feeding of larvae account for the greatest amount of soft tissue destruction in the decomposition process (Mann et al., 1990). It has been observed that some of the factors affecting the rate of decomposition also indirectly affect the progression of insect colonisation, growth, and feeding (Galloway et al., 1989; Mann et al., 1990; Campobasso et al., 2001).

Arthropod succession of a carcass can vary between any given habitat (Smith, 1986). The general community of invertebrates present on decomposing carrion comprise of flies (*Diptera*) and beetles (*Coleoptera*). However, other orders can be present from wasps and ants (*Hymenoptera*), spiders, scorpions, mites, and ticks (*Arachnida*), cockroaches (*Blattaria*), and millipedes (*Diplopoda*) (Castner, 2010). These insects can be further divided into their feeding relationships: necrophages (feeding exclusively on decomposing tissue), predators and parasites of necrophages, omnivores (feeding on the decaying flesh and insects inhabiting the remains), and opportunists (arrive on remains due to proximity to their local environment) (Goff, 1993). Specific insects are associated with different phases of decomposition (Clark et al., 1997).

The fresh stage of decomposition is common for insect invasion of the first wave colonisers, *Calliphoridae* (blowflies), *Sarcophagidae* (flesh flies), and *Muscidae* (house flies). These species are brought on by the bacteria-generated semiochemicals released from the orifices of the carcass. The female flies will lay their eggs on the body with oviposition typically associated with the mouth and anus; however, this depends on the body's exposure, i.e., clothing or wrapping (Kelly et al., 2008; Goff, 2009). The larvae of the flies are the initial consumers of flesh and can reduce a carcass to skeletonisation in two weeks (Davis & Goff,

2000). If insect access to the carcass is delayed or postponements in maggot colonisation is brought on by harsh weather, e.g., rain and high winds preventing oviposition, there will be fewer maggot colonies, which will result in slower decomposition rates (Mann et al., 1990; Kelly, 2006). During the active decay stage of decomposition, perforation of the abdomen heralds the second wave colonisers including, *Diptera* larvae and carnivorous beetle colonies (*Staphylinidae, Silphidae* and *Hiteridae*) (Goff, 2009). The carnivorous beetles are seen to arrive once the maggot colonies have been established; their arrival too early can adversely affect the formation of maggot populations, consequently slowing the rate of decomposition (Forbes & Dandour, 2010). As decomposition progresses and the tissue of the carcass becomes desiccated, the third wave of colonisers inhabit the remains in the form of cheese skippers (*Piophilidae*) and the *Coleoptera* species, hide beetles (*Dermestidae*), chequered beetles (*Cleridae*), and skin beetles (*Trogidae*) (Smith, 1986). These colonisers are responsible for moving the carcass to skeletonisation. For example, hide beetles can significantly alter the rate of skeletonisation by reducing remains in as few as 24 days (Castner, 2010).

If insects are hindered from access to the carcass, the rate of decomposition would be greatly decreased. The latter observations warrant researchers to refer to the entomological research to interpret and make inferences about the effects of the different factors on the rate of decomposition, as insect activity is highly influenced by the environment the remains are found in. However, when analysing scavengers involved in the process of decomposition, another group is far quicker in reducing a carcass to skeletonisation than the insect population, namely vertebrate scavengers.

Vertebrate scavengers

Like insects, vertebrate scavengers will consume carrion if access is not hindered. Vertebrate scavengers can reduce a carcass to skeletonisation in a matter of hours (Reeves, 2009; O'Brien et al., 2010). Larger scavengers can be responsible for the rapid removal of biomass (skin and muscle tissue) resulting in an increased rate of decomposition. As seen in Cape Town, South Africa by Spies et al. (2018), scavenger activity was present throughout the entire experimental phase. The Cape grey mongoose reduced pig carcasses to skeletonisation in 14 days compared to the control carcass remaining in advanced decomposition for 93 days (Spies et al., 2018). Similar scavenger assemblages are present in Hawaii (Dibner et al., 2019), with the yellow mongoose present around the pig carcasses, however, the mongooses were feeding on the invertebrate scavengers present on the body delaying the subsequent insect colonisation. The

presence of large vertebrate scavengers is noted from the fresh stage until skeletonisation, the scavengers are more present until the carcass enters the advanced stage of decomposition and the remaining tissue starts to desiccate (O'Brien et al., 2010).

Different geographical regions have differing forensically relevant scavenger guilds, e.g., South African large scavengers include vultures, black-backed jackal, domesticated dogs, and meercats, wild dogs, lion, leopard, porcupine, and hyena (Keyes et al., 2019; Keyes et al., 2021), Massachusetts, United States of America scavengers include vultures, racoon, and opossum (Pokines & Pollock, 2018). The differing guilds are representative of the differing climatic variables and the regional specific species (O'Brien et al., 2010)

Not only do scavengers increase the rate of loss of biomass, but they are also involved in the scattering of remains, which may hinder the process of victim identification (Spies et al., 2018). Possible confusion can be caused if scavenger activity is misinterpreted as trauma (Keyes et al., 2019). Therefore, consideration of possible taphonomic effects of scavengers needs to be considered when estimating the PMI (Mann et al., 1990; O'Brien et al., 2007).

Temperature

Tsokos (2005) stated, as a rule, a change in the environmental temperature will result in a change in the rate of decomposition, however not changing the underlying biological process of decomposition post-mortem. The greatest influencer on the rate of decomposition is ambient temperature. The temperature can start, control, and halt many biological processes (Mann et al., 1990; Vass et al., 1992; Shean et al., 1993; Megyesi et al., 2005; Finaughty & Morris, 2019). Temperature is one of the biggest factors to consider between seasons as it will affect the rate of decomposition of carrion, insect activity, and insect growth (Gill-King, 1997). The increased temperature speeds the chemical reactions and increases the insect larvae's growth and feeding rate (Shean et al., 1993).

Seasonal changes in insect activity are observed where flies would only lay eggs under certain conditions including sufficient sun exposure during cooler seasons and protection in the form of shade during extremely hot seasons, ensuring the survival of maggot colonies (Galloway, 1997). Shean and colleagues (1993) highlighted that the rate of decay is slower when there is less solar exposure and a lower ambient temperature. Research in Western Cape, South Africa has shown that there is a turning point where the effects of sun exposure cause the process of decomposition to stall and preservation to take place (Finaughty & Morris, 2019). However,

when increased temperatures are combined with increased humidity or a possible aquatic environment, the progression and rate of decomposition can be altered. Temperature as a factor affecting the rate of decomposition cannot always be isolated from other factors, such as humidity and rainfall, which are dependent or co-dependent on temperature (Galloway et al., 1989; Mann et al., 1990). The factors play a combined role in the rate of decomposition along with insect and bacterial activity (Finaughty & Morris, 2019).

The temperature where biological processes are noted to stop is called the base temperature (Megyesi et al., 2005). The base temperature for decomposition has not yet been confirmed, with different values having been suggested by forensic entomologists as 10°C or 6°C dependent on fly species (Catts, 1992), forensic anthropologists' suggesting 0°C (Vass et al., 1992) and below 4°C, as bacterial growth is then hindered (Micozzi, 1991). Mann et al. (1990) reported fly eggs and larvae to die when temperatures outside of the body reached 0°C, but if located inside the body, the colony would produce enough heat to sustain life if numbers are high enough. Freezing of matter is considered the most common form of preservation. Any temperature below 0°C is incompatible with biological processes. Therefore, researchers have commonly agreed that decomposition is almost completely halted at 0°C (Micozzi, 1991; Megyesi et al., 2005).

In colder conditions, autolysis and putrefaction are delayed and the onset of insect activity is only noted towards the end of advanced stages of decomposition (Meyer et al., 2013). This results in a slower decomposition rate in colder temperatures and faster decomposition rates in warmer temperatures. When remains have been frozen and start the process of thawing, the pattern of decomposition is seen to alter from the typical fresh body decomposition (Micozzi, 1997). Frozen-thawed remains display decay more externally, with the process of autolysis of external tissue resulting in greater insect colonisation and access of soil organisms and aerobic bacteria. In contrast, fresh tissue displays a greater initial breakdown of internal tissue due to putrefaction. Therefore, frozen-thawed remains display an "outside-in" pattern of decomposition and a fresh body displaying an "inside-out" pattern of decomposition (Micozzi, 1986). This "outside- in" pattern of decomposition may alter the interpretation of the PMI resulting in an incorrect estimation of time since death.

Humidity/Aridity

As climates change from region to region, so does the moisture content of the air (Jaworski & Hilszczański, 2014). In an environment that is extremely humid, decomposition can be stalled with adipocere formation in the body. Remains in an arid environment may desiccate due to the lack of moisture (Mann et al., 1990; Clark et al., 1997; Vass, 2001). Mummification as a result of desiccation will halt the rate of decomposition completely. The presence and absence of moisture can act as a variable that influences the rate decomposition to stall and in certain cases increase the rate of decay.

A study in Tennessee, United States of America, conducted to observe seasonal differences between the rate of decomposition found that although autumn remains experienced lower temperatures than the summer deployments, the rate of decomposition and the TBS was not significantly different during the early stages of decomposition. Upon comparing other environmental factors, the humidity levels were relatively similar during summer and autumn (Giles et al., 2020). Giles et al. (2020) proposed that humidity was the overarching driving force in the earlier stages of decomposition during both seasons.

Humid environments generally have an increased rate of decomposition due to sufficient moisture levels for larvae's survival and insect activity (Galloway et al., 1989; Mann et al., 1990). If remains are in a closed structure, it has been seen to decay faster as humidity is not released from the space and temperature will be maintained which accelerates the bacterial and autolytic processes. If such enclosed environments are ventilated, and the humidity is removed through evaporation from the environment, remains can become mummified due to the inhibition of bacterial and insect activity (Galloway et al., 1989; Campobasso et al., 2001).

Rainfall

Human remains located above ground are subject to all environmental elements that affect the rate of decomposition, including rainfall. Mann et al. (1990) concluded rainfall to be a variable that affects the rate of decay. Research looking solely at the effects of rainfall and rehydration on the rate of decomposition is limited, due to the difficulty of isolating the subsequent effects. Effects of rainfall are frequently described as part of a general overview of the seasonal impact on the decomposition process. Most of the research focuses on the effects of a lack of moisture and its impact on rates of decay (Archer, 2004; O'Brien et al., 2007; Marella et al., 2013; Mehendiratta et al., 2015; Finaughty & Morris, 2019; Forbes et al., 2019). Topics to be

explored within this section include types of rainfall/rehydration and effects on rate of decay and insect activity, links between other environmental factors, and finally, what effect does rainfall/rehydration play on the rate of decomposition?

The presence of rain is said to speed up decomposition through possible liquefaction of remains or slowing it down by washing away or reducing invertebrate scavengers and decreasing the ambient air temperature (Mann et al., 1990). The ambient temperature is also linked to the presence of humidity, the two are directly proportional with a rise in humidity displaying a rise in ambient temperature. The increased humidity is linked to the increased insect activity and results in an increased rate of decomposition (Giles et al., 2020). The impact of rainfall was summarised by Mann et al. (1990) to have little effect on maggot activity during early decomposition. However, the fly activity would be affected/halted during moderate to heavy rainfall, hinder insect activity, and may drown *Diptera* eggs and larvae reducing the viable populations (Mann et al., 1990; Anderson & VanLaerhoven, 1996). Heavy rainfall has been reported to have no impact on the mechanical breakdown of the decomposed tissue (Mann et al., 1990).

There has been little to no research looking at the effects of different types of rainfall on the rate of decomposition. The latter includes hard pelting rain, constant rainfall, light rainfall and when in the decomposition cycle it occurs. The timing of rainfall within the decomposition process could be seen to alter the rates of decomposition, as mentioned should rain fall before maggot populations are established this would then adversely affect the rate of decay by disrupting the subsequent insect colonisation and delay carrion feeding (Mann et al., 1990; Kelly et al., 2009).

Rainfall can also be correlated with a drop in temperature and causes the soil to become moist, therefore not allowing remains to dry out, which will promote bacterial growth within the remains resulting in an increased rate of decay (Archer, 2004). However, a drop in temperature is linked with decreased insect activity which can decrease the rate of decomposition (Megyesi et al., 2005). The effects of rainfall have not been statistically separated from temperature (Archer, 2004), as separating the impact of these variables on insect activity and the rate of decomposition is difficult. The combination of higher temperatures and rainfall are associated with a greater rate of decay. However, if the remains have dried out prior to rehydration during the winter/dry months, the PMI estimation may be inaccurate due to the assumption that the remains just decayed faster because of the high temperatures and rainfall. Moreover, it would

mask the "stagnant period" and estimate a PMI much shorter than the actual PMI (Myburgh et al., 2013).

A study conducted by Myburgh and colleagues (2013) noted that remains that entered the dry phase of decomposition, also known as desiccation, exhibited slowing of decomposition following periods of no rain during the winter months. Faster rates of decomposition (TBS above 17) were observed in the summer months (Myburgh et al., 2013). The winter decomposition showed a sigmoid curve of TBS, where initially the decomposition was rapid and then slowed down to the point of nearly stopping. Active decomposition continued again after an extended period of inactivity. This suggests that temperature and humidity might not be the only factor playing a role in the rate of decomposition, and it was proposed that rainfall might also be playing a role (Myburgh et al., 2013).

It is therefore important to establish the effects of rainfall/rehydration on remains that have dried out and stalled in the advanced decomposition phase. Smith (1986) found that sometimes dried remains can be re-colonised by maggots after rehydration by rainfall. Forbes et al. (2014) observed this as a trend and speculates this to be the cause for re-initiated decomposition after rain was present. Rainfall has been revealed to rehydrate desiccated tissue to a moist decomposition phase (Ayers, 2010). Ayers (2010) further observed desiccated remains, exposed to heavy rain, rehydrating, and restoring the fresh appearance. Although no blowflies were seen to return to the remains, but rather “unknown” scavengers returned to feed on the previously desiccated remains (Ayers, 2010).

To the best of the authors' knowledge, no study has been conducted to test the direct effect of rehydration (rainfall) on the rate of decomposition. Creating an experimental design to control for rainfall is very challenging, with the attempt to delineate what effects are contributed solely to rainfall or the myriad of factors that affect the rate of decomposition. The difficulty with rain is the fact that seasons differ in the timing of rainfall. For example, in South Africa, we have winter rainfall at the coast and summer rainfall inland, and the remains are also acted upon by regional-specific climatic differences (Dyson, 2009). The Highveld region of South Africa experiences an average annual rainfall of 500 mm – 900 mm (Conradie, 2012; South African Weather Services, 2022), with most of the rainfall occurring during the summer months (December – March). These climatic differences are the drivers of insect progression. Since insects are a major driver of tissue breakdown in carrion, it is essential to consider as many factors as possible to understand how their activity is influenced thoroughly.

Therefore, it is proposed that once decomposition has stalled in the advanced stage, rainfall is the reason for rehydration of the remains. The rehydration allows for the progression past the point of desiccation, when compared to remains that stall in a state of desiccation and are not exposed to rain. The success of rehydration could potentially re-attract insects and allow insects to continue breaking down the tissue as it has become palatable once again (Forbes et al., 2014). Thus, if we can understand the effects of rehydration on the rate and progress of decomposition, we can better understand the effects that it has on the PMI, regardless of the ADD and as such potentially justify the need for rain/rehydration to be incorporated into the PMI estimation methods, and not only temperature as is current practice (Megyesi *et al*., 2005).

Chapter 3 Materials and methods

3.1 Experimental location

This study was conducted at the Forensic Anthropology Body Farm (FABF) on the Miertjie Le Roux Experimental Farm, University of Pretoria. The farm consists of 570 hectares (5.7 km^2) in the Cullinan District, Gauteng Province and is in the Central Highveld plateau of South Africa (Myburgh et al., 2013). The experiment took place in a fenced-off section of the farm, which is approximately 0.5 hectare (5000 m^2), with an access gate to allow for placement of pig carcasses (Figure 3.1). The fence of the FABF limits access of local wildlife (Figure 3.1), however does not block it completely. Therefore, cages (Figure 3.2) were used in this study to cover the pig carcasses during the progression of decomposition. The cages, therefore, prevented access to larger vertebrate scavengers known to roam in the area, such as wild dog (*Lycaon pictus*) and black-backed jackal (*Canis mesomelas*) (Keyes et al., 2020).



Figure 3.1: The FABF on the Miertjie Le Roux Experimental Farm, University of Pretoria, illustrating the 0.5 hectare fenced off enclosure with an entrance gate (indicated with a white arrow) (Google Maps).

The cages were custom designed for the purpose of this study. Each cage measured 0.9m x 0.9m x 2.0m and was made with a metal frame lined with a mesh (chicken/bird mesh wire) to prevent the entry of large scavengers. The top of each cage was also covered with a transparent plastic tarp to protect the pig carcasses from direct rainfall. The cage was constructed to the length and breadth of an adult pig and to allow for sufficient coverage (provided by the transparent tarp) from natural rehydration. The base of the cage was left open for the carcass to have contact with the ground to allow air and insect access and as not to hinder the decomposition process within the parameters of this study. The cage was lifted, as per the demonstration in Figure 3.2, when observation of the carcass took place and while scoring TBS.

The vegetation of the study site mainly consists of Highveld grassland and sour veldt grasslands or sour bushveld (Acocks, 1988; Grobler et al., 2006) (Figure 3.2). The Köppen climate classification of this region is Cwb; (C) Temperate, (w) dry winter, (b) warm summer (Conradie, 2012). Rainfall mainly occurs during the summer months in this area, with limited winter showers (South African Weather Services, 2022).



***Figure 3.2*:** Custom metal cages, 0.9m x 0.9m x 2.0m, surrounded with chicken mesh wire to prevent the entry of large scavengers and covered with a transparent plastic tarp, fastened to the cage, to prevent natural rehydration.

3.2 Materials

3.2.1 Pigs

A total of 12 domestic pig (*Sus scrofa domesticus)* carcasses (deceased remains), weighing approximately 40-100 kg, were used in this study. Ethical clearance for the use of animal material in this study was obtained from the University of the Witwatersrand Animal Ethics Committee (AESC20-05-003; 2020/05/05/O) and the University of Pretoria Research Ethics Committee (REC104-20) and a Section 20 approval was granted by the Department of agriculture, land reform, and rural development (see Appendix A, B, & C). The specific weight of the pig carcasses were chosen to represent the average weight of an adult human, as the body size and weight of a carcass have been shown to affect the rate of decomposition (Sutherland et al., 2013). The pig carcasses were collected from G. H. Braak Farm (GHB Farm) in Rooipoort, Gauteng and placed within a day of death. Pigs collected within one day after death were still fresh with reduced signs of physical decay. Only pig carcasses with no external trauma were accepted for this research, as an increase in invertebrate colonisation was observed in cases of trauma or external wounds, which accelerated the rate of decomposition in a previous study (Kelly et al., 2009). Furthermore, only pigs that died of natural causes were used. The pigs mainly died from diseases such as *Lawsonia intracellularis* and *Haemophilus parasuis,* common amongst pig farms (Keyes et al., 2019). Although disease effects the rate of decomposition of carrion (Campobasso et al., 2001), all the pigs were sourced from the same farm and therefore are exposed to similar conditions, which reduces differences between the sample population.

A pig model was used due to the similarities between human and pig decomposition and the similarities with regards to the skin, weight, diet (omnivorous) and intestinal flora (Stokes et al., 2013). Advantages for the use of pig's carcasses are body mass, body composition, muscle to fat ratio, and gross process of decay that are similar to those of human bodies (Matuszewski et al., 2019). The model allows for uniformity of the experimental sample, statistical rigor, and with the legal and ethical challenges prohibiting the use of donated remains in forensic taphonomic studies in many countries, it provides ease of access to an already accepted bred and domesticated species. Pigs are still not the perfect proxy for human decomposition studies, due to the difference in limb proportions and diet. This does present limitations to the interpretation of the results yielded from these experiments as the timelines and rates cannot be directly applied to human forensic cases (Miles et al., 2020). Nevertheless, pig models

provide valuable information about trends that could be seen in validation studies where human cadavers are not available (Miles et al., 2020). Also, the use of this human-analogue allows for greater replication and the control of various confounding factors that affect the rate of decomposition (Matuszewski et al., 2019).

3.3 Methods

3.3.1 Total body scoring

Decomposition scores were collected two to three times a week until the carcass reached the advanced stage and there-after once a week until remains had dried in the advanced stage of decomposition. The criteria for reaching the advanced stage of decomposition were a TBS score that has not changed for three site visits. On the third visit of the TBS score remaining the same, the experimental carcass was rehydrated. The TBS was collected during each visit, using the adapted description and scores proposed by Keough et al. (2017) when using a domestic pig (*Sus scrofa domesticus)* sample. Each section of the body was assessed according to the delineation of body regions described in Myburgh et al. (2013) and Keough et al. (2017) (Figure 3.3), including: head and neck – from snout to start of shoulder girdle; trunk – from shoulder girdle, thorax, abdomen, and pelvic girdle; and limbs – where the limbs meet the trunk, excluding the shoulder and pelvic girdles. The total of the points from each region shown in Table 3.1 – 3.3 were added together and the sum of the points gave the TBS value for that stage of decomposition (Megyesi et al., 2005).

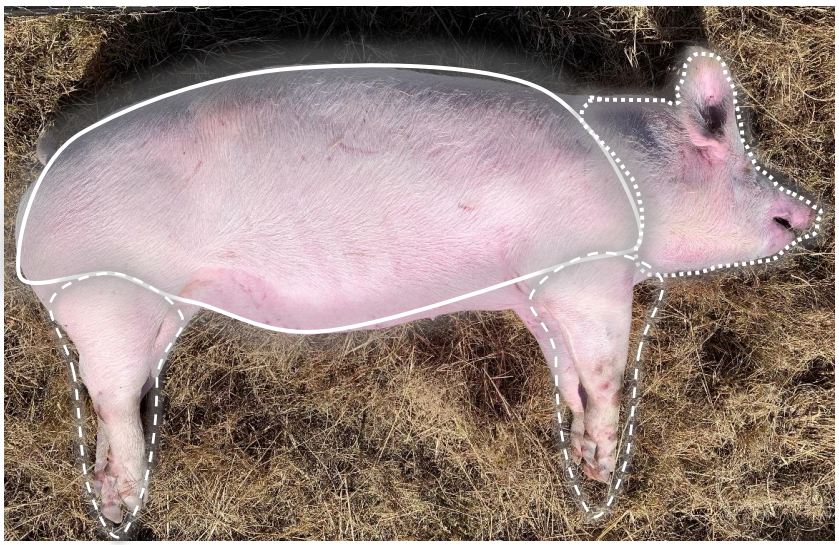

Figure 3.3: Regions of the body, including the head and neck (dotted line), trunk (solid line) and limbs (dashed line) assessed to establish the TBS following guidelines as set out by Keough et al. (2017).

Tables 3.1 – 3.3 summarises the scoring descriptions and show the visual representation of the corresponding qualitative description associated with the quantitative TBS values used for scoring, as per the stipulations by Keough et al. (2017).

Table 3.1: Qualitative descriptions and visual representations of TBS values for the head and neck region (adapted from Keough et al. (2017))

Stages and descriptions for <u>head and neck (pigs)</u>	**Point**	**Photograph**
1. Fresh 1.1 Fresh, no discolouration with signs of slight lividity (pink/red)	1	
2. Early decomposition 2.1 Insect activity with pronounced lividity (dark pink/red)	2	
2.2 Dark red discolouration with some flesh still relatively fresh; oedema of ears; maggot colonisation (mouth); initial bloating of neck and skin slippage	3	

Stages and descriptions for <u>head and neck (pigs)</u>	Point	Photograph
2.3 Discolouration and/or brownish shades particularly at edges, drying of nose, ears, and lips; prominent bloating of neck; maggot colonisation (mouth and eyes); purging of decompositional fluids (mouth)	4	
2.4 Purging of decompositional fluids (mouth, eyes, nose); brown discolouration; hair loss and skin slippage; drying of lips, nose, and ears	5	
2.5 Black discolouration of flesh; extensive maggot colonisation and migration	6	

Stages and descriptions for <u>head and neck (pigs)</u>	Point	Photograph
3. Advanced decomposition 3.1. Caving in of the flesh and tissue of eyes and throat	7	
3.2. Moist decomposition with bone exposure less than one half of that area being exposed	8	
3.3. Desiccation with bone exposure less than one half that of the area being score	9	

Stages and descriptions for <u>head and neck (pigs)</u>	Point	Photograph
4. Skeletonisation 4.1 Bone exposure of more than half of the area being scored with greasy substances and decomposed tissue	10	
4.2 Bone exposure of more than half of the area being scored with desiccation or mummified tissue	11	
4.3 Bones largely dried, but retaining some grease	12	
4.4 Dry bone	13	
Total		

Table 3.2: Qualitative descriptions and visual representations of TBS values for the trunk region (adapted from Keough et al. (2017))

Stages and descriptions for <u>trunk (pigs)</u>	Point	Photograph
1. Fresh 1.1 Fresh, no discolouration – slight lividity (pink)	1	
2. Early decomposition 2.1 Skin appears shiny/glossy with early bloating and may show purple-black discolouration over abdominal area	2	
2.2 Purple-black discolouration and purging of decompositional fluids; skin slippage with maggot filled blisters present; hair loss	4	
2.3 Post-bloating following release of abdominal gases, with extensive skin slippage and drying out of blisters	5	
3. Advanced decomposition 3.1 Decomposition of tissues producing sagging of flesh; caving in of abdominal cavity	6	

Stages and descriptions for <u>trunk (pigs)</u>	Point	Photograph
3.2 Moist decomposition with bone exposure less than one half of that area being exposed	7	
3.3 Desiccation with bone exposure less than one half of the area being score	8	
4. Skeletonisation 4.1 Bone with decomposed tissue, sometimes with body fluids and grease still present	9	
4.2 Bone with desiccated or mummified tissue covering less than half of the area being scored	10	
4.3 Bones largely dried, but retaining some grease	11	
4.4 Dry bone	12	
Total		

Table 3.3: Qualitative descriptions and visual representations of TBS values for the limb region (adapted from Keough et al. (2017))

Stages and descriptions for <u>limbs (pigs)</u>	Point	Photograph
1. Fresh 1.1 Fresh, no discolouration with slight lividity (pink) and rigor present	1	
2. Early decomposition 2.1 Pink-white bloating appearance with bloating of proximal parts of limbs	2	
2.2 Grey to green discolouration; marbling and shiny appearance of skin; some flesh still relatively fresh; skin slippage and hair loss	3	

Stages and descriptions for <u>limbs (pigs)</u>	Point	Photograph
2.3 Discolouration and/or brownish shades particularly at edges, drying of skin (starting distal to proximal)	4	
2.4 Brown to black discolouration, skin having a leathery appearance	5	
3. Advanced decomposition 3.1 Moist decomposition with bone exposure less than one half of that area being exposed	6	

Stages and descriptions for <u>limbs (pigs)</u>	Point	Photograph
3.2 Desiccation with bone exposure less than one half that of the area being score	7	
4. Skeletonisation 4.1 Bone exposure over one half of the area being scored, some decomposed tissue and body fluids remaining	8	
4.2 Bones largely dried, but retaining some grease	9	

Stages and descriptions for limbs (pigs)	Point	Photograph
4.3 Dry bone	10	
Total		

3.3.2 Deployment

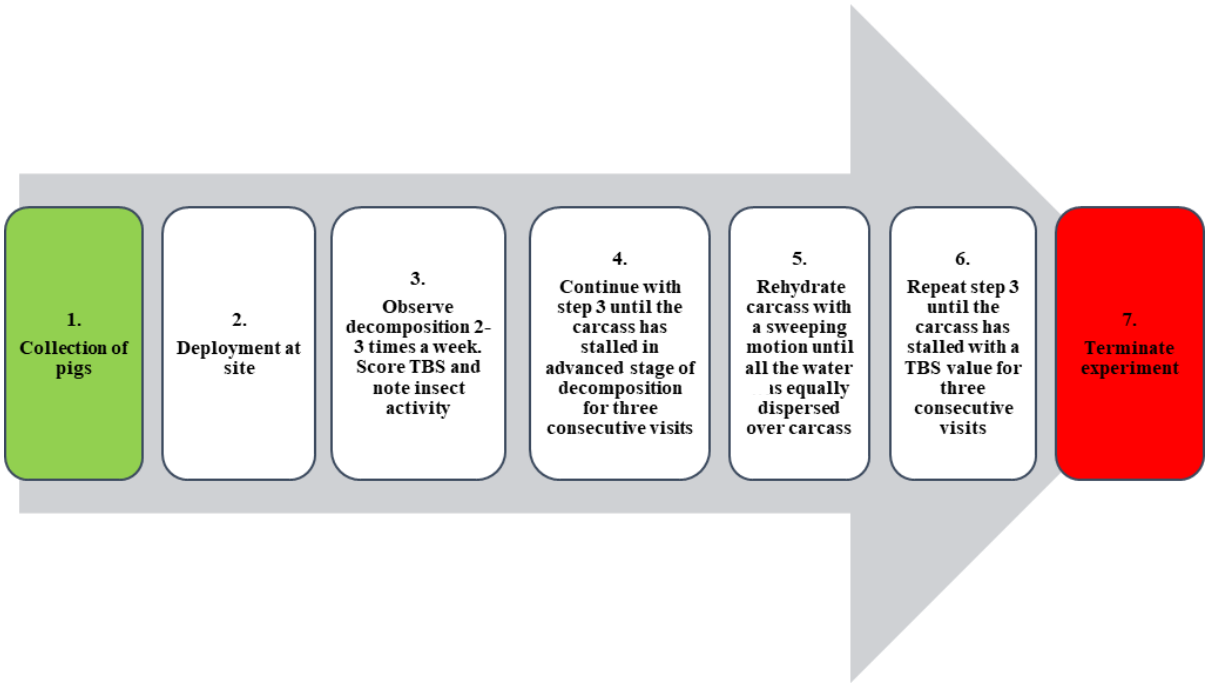

Figure 3.4: Experimental design for deployment, scoring and rehydration of pig carcasses.

The carcasses were collected from the farm the morning of deployment and transported to the experimental farm where it was then deployed at the FABF site. Four deployments took place with three pigs deployed each time (April, July – August, and early September 2021). In three instances, only two fresh carcasses were available for the deployment and therefore the

additional third carcass could not be deployed on the same day, but was deployed within the same week as the other corresponding carcasses. Of the three carcasses deployed each time, one carcass served as the control (CP) and the other two served as the experimental (EP) carcasses which were rehydrated. At minimum, there were two pigs deployed at one time, a control, and an experimental carcass and a second experimental carcass was then deployed within the same week, as described above. A pilot pig carcass was deployed, prior to the commencement of the study, in order to determine how frequently the carcasses needed to be scored, when the carcasses need to be rehydrated, and to establish a rehydration protocol (Figure 3.4).

After trial and error of a pilot deployment of a pig carcass, the following protocol for controlling and measuring external factors were established and implemented.
Each pig carcass was deployed in a cage with a transparent tarp covering. Initially three cages were constructed for Trial 1 due to time and budget constraints (Figure 3.2). A further three cages were later constructed, allowing six pig carcasses to be deployed at once for Trials 2 and 3. In order to increase the sample size, an additional three secondary cages were constructed for Trial 4 (Figure 4.2). These cages were constructed to hinder larger vertebrate activity while allowing invertebrate access and to prevent rain from falling directly onto the carcasses (Figure 4.1). The secondary cages were constructed with metal rods and chicken mesh wire and covered with a plastic tarp (Figure 4.2).

3.3.3 Rehydration

Rehydration only took place once the carcass had stalled in advanced stage of decomposition for three consecutive visits. Rehydration involved the use of a watering can filled with prepared water (artificial rehydration). Prepared water was chosen due to availability and to resemble natural rehydration (rain), which is typically devoid of excessive chemicals used in the general water supply (e.g., high chlorine levels). The water obtained for the study was sourced from OASIS©, which has gone through reverse osmosis with the lowest total dissolved solids when compared to other leading brands in South Africa (Kraaij, 2010). Rainwater has a pH of between 5.0 and 5.5 and pure prepared water/distilled water has a pH of 7. However, distilled/prepared water, after being exposed to the atmosphere, absorbs carbon dioxide, and reduces the pH to a slight acidic 5.8 (Deziel, 2018). Therefore, it was concluded that prepared

water would serve as an adequate substitute for rainwater. The use of artificial rehydration was chosen to control for rehydration events otherwise impossible with natural rehydration and allow for the timing of the rehydration events to be controlled for. The experimental carcasses were rehydrated with an amount of water that is equivalent to the average rain (10 mm = 10 L per square meter) that falls during a typical rainstorm in the Highveld region (Dyson, 2009). An area of one square meter was marked out around the carcass, and the water was poured over the area where the carcass lay (Figure 3.5). Thereafter the rate of decomposition was followed by scoring the TBS, until the end of the experimental period. Criteria for termination of the experiment involved the carcasses reaching skeletonisation for the control carcass, and/or a state of stasis in the TBS score for both the control and experimental carcass for 3 consecutive visits.



Figure 3.5: Method of watering carcass once stalled TBS in the advanced stage of decomposition.

3.4 Data collection

After capturing the TBS values as per the stipulations of Keough et al. (2017), general observations were also noted for each carcass. These observations included what major changes had occurred in comparison to the previous visit i.e., scavenger activity, insect activity, state of carrion tissue, bone exposure. A series of photographs were taken with an iPhone XR 12-megapixel wide angle camera. A photograph was taken of each region of the carcass: head and neck, trunk, and limbs (front and hind limbs) and a full body shot. Photographs were also taken of any notable variations observed on the carcasses and of the different insects that colonised the remains. The presence of insects was recorded when assessing each carcass in terms of presence or absence and type of invertebrate present. The type and location of the insects on the carcass was also noted, i.e., head and neck, trunk, or limbs from photographs taken at the site and compared to insect identification charts (Finaughty, 2019). The skeletal remains are to be used in a longitudinal research study, currently obtaining ethics for the use of the dry bones that remains protected at the research site.

Weather data was obtained from World weather online (2022) directly in the form of a raw excel sheet containing; wind speed (m/s), temperature (°C), rainfall (mm), pressure (hPa), and humidity (%) from the Rayton weather station nearest to the FABF (World weather online, 2022.).

3.5 Data Analysis

The TBS was used to measure decomposition, with scores collected three times a week over the experimental period and once a week after the advanced stages of decomposition were reached. In order to standardise and compare the TBS values for each pig, ADD were calculated with the sum average daily temperatures, once the experimental phase was completed. The ADD was calculated by adding the average daily temperatures from day one of the deployment per pig carcass until day of termination. ADD is used as a measurement of the thermal energy the carcass was exposed to during the decomposition cycle (Megyesi et al., 2005). ADD is common measure between the carcasses and allows for the direct comparison, regardless of the day of deployment date of each carcass. ADD alone is not an accurate measure for PMI estimations as seen in the Western Cape (Forbes et al., 2019), and Highveld area in South Africa (Myburgh et al., 2013). However, ADD is one factor that is constant between all experiments and assists in the analysis of other variables by limiting the effect of temperature

between deployments even though seasonality may still differ. The seasonal accuracy has yet to be successfully applied (Myburgh et al., 2013), and the inclusion of other factors (such as rainfall) in the regression formulae for PMI estimation relies on findings showing that rainfall is a significant factor in the rate of decomposition and can be incorporated into the formula.

The rate of decomposition was represented in graphic form. The TBS was plotted against ADD in a scatter plot for both the control and the experimental pigs in order to determine whether notable differences occurred in the progression of decomposition. The data collected was categorised into cooler and warmer trials. The data was analysed for each set of four trials and compared based on temperatures experienced during the experimental phase. The separation was due to the definite separation of two main deployments, one in the colder month of April, the other three in late July – early September.

Insect activity was noted for three regions of the body (head and neck, trunk, and limbs). Retrospective analysis from photographs and site notes was done by assigning a score of 0 for absence and 1 for presence of insect activity for the three body regions respectively for every site visit. The scores were totalled for every visit, with a score of 1 represented insects' presence in only one region of the body, 2 represented insect activity in any 2 regions, and 3 represented insect activity in all three regions of the body. The regional scores represented insect activity over the experimental phase plotted against ADD as a time line.

Intraclass correlation was performed in order to determine the repeatability of the method of scoring TBS. Inter-rater and inter-rater reliability was assessed by the primary investigator and an independent assessor through photographs taken during each visit. The assessment took place during the data collection period, three months after the end of the first deployment (experimental pig 1 (EP1) and control pig 1 (CP1)). The primary investigator re-scored the TBS for EP1 and CP1. The independent assessor was provided with a full set of catalogued photographs and re-scored the TBS. An intraclass correlation (ICC) was performed on IBM SPSS to determine the repeatability of the scoring methods used to assess decomposition (Allan, 1982; Anderson 2010).

Chapter 4 Results

The main aim of this thesis was to address the effects of rehydration on decomposition using a pig model. This longitudinal study took place over eight months, with the first deployment on 21 April 2021. The study period took place over the cooler autumn, winter, and early spring months of April to November (Winter solstice 21 June 2021) in the Highveld region of Gauteng, South Africa. The entire experimental phase was completed on 12 November 2021, totalling 206 days (ADD 3719), with four control and eight experimental pig carcasses.

4.1 Climatic conditions and study sample

The deployments of the study pigs were spread over the trial period (Table 4.1, 4.2, & 4.3). The deployment dates resulted in two distinctive placements, one cooler (Trial 1) and three warmer (Trial 2, 3, and 4) deployments based on the maximum temperatures experienced. The temperatures experienced between the two sets of deployments differed by an average daily temperature of ±4 °C and experienced differing maximum temperatures of ±9°C (Table 4.2). Over the study period, the approximate minimum temperature was 2°C, a maximum temperature of 36°C, a mean average daily temperature of approximately 18°C, and a total of 45.6 mm of rain (World weather online, 2022).

The pig carcasses were collected based on availability at the G.H. Braak pig farm, Rooipoort. The pigs were only accepted for the research if they fell within the weight range of 40-100kg (Table 4.3). The carcass weights were an estimated average of 65kg as seen in Table 4.3. The sex of the pigs was also randomised based on availability from the pig farm. The assignment of experimental and control pig was also randomised and allocated upon deployment.

The TBS was noted and calculated for each pig after site visits. Using IBM SPSS an ICC value was calculated for CP1 and EP1, respectively. The intra-rater reliability values for CP1 and EP1 were 0.991 and 0.961, while the inter-rater reliability values were CP1 and EP1 to be 0.979 and 0.960, as seen in Appendix F.

Table 4.1: Comparison of minimum, maximum, average daily temperature, and total rainfall for Trial 1, 2, 3 and 4 over each experimental period respectively, including month of deployment.

Temperature (°C)	Trial 1 (April)	Trial 2 (July)	Trial 3 (August)	Trial 4 (September)
Minimum	2	2	4	10
Maximum	27	36	36	36
Daily average	15.4	19.9	21.1	21.9
Total rainfall (mm)	22.2	23.4	23.4	22.3

Table 4.2: Comparison of average daily temperature, maximum and minimum temperature for the cooler and warmer trials.

Temperature (°C)	Cooler trial	Warmer trials
Minimum	2	2
Maximum	27	36
Daily average	15.4	19.85

Table 4.3: Summary of the cooler and warmer deployment, descriptive statistics related to the pig samples as well deployment/death, rehydration, and termination dates.

Deployment	Trial #	Pig	Sex	Weight (kg)[a]	Deployment (date)	Rehydration (date)	Termination (date)
Cooler	**1**	EP1	Female	60	2021/04/21	2021/05/24	2021/07/27
		EP2	Female	70	2021/04/23	2021/05/24	2021/07/27
		CP1	Female	60	2021/04/23	-	2021/07/27
Warmer	**2**	EP3	Female	90	2021/07/21	2021/10/11	2021/11/12
		EP4	Male	40	2021/07/21	2021/09/21	2021/11/12
		CP2	Female	40	2021/07/21	-	2021/11/12
	3	EP5	Female	50	2021/08/13	2021/09/21	2021/11/12
		EP6	Male	100	2021/08/18	2021/10/11	2021/11/12
		CP3	Male	50	2021/08/13	-	2021/11/12
	4	EP7	Female	80	2021/08/31	2021/10/01	2021/11/12
		EP8	Male	70	2021/09/02	2021/10/01	2021/11/12
		CP4	Male	70	2021/08/31	-	2021/11/12

[a] Estimated



Figure 4.1: One of the six primary cages constructed for the purpose of preventing scavenger activity and direct rainfall. Photographic record of cage and tarp after rain at experimental sight.



Figure 4.2: Secondary cages, constructed with mesh and iron rods covered with a plastic tarp. **A**) A side profile displaying cage breadth and height, and **B**) front profile of cage displaying cage width and height and connection of tarp with space for airflow.

4.2 Decomposition

An objective of this research was to assess the effects that rehydration had on the rate and progression of decomposition. The data collected were grouped in their respective trials for ease of analysis. In order to understand the effects that rehydration had on the rate of decomposition, the initial results were based on decomposition until the point of rehydration. Assessments were then conducted on post-rehydration and the effects on the rate of decomposition. The analysis was grouped into cooler and warmer deployments as described above with the cooler deployment consisting of Trial 1. The pigs of Trial 1 were kept in a cold room overnight to allow for multiple pig deployments, all on the same day. The warmer deployment consists of Trials 2, 3, and 4, with overlapping weather conditions (Table 4.1, 4.2, & 4.3). The progression and rate decomposition, insect activity, and climatic conditions were assessed and compared for both cooler and warmer deployments.

4.2.1 Cooler trial

Trial 1

The carcasses of the pigs in Trial 1 were deployed in the cooler months of the year. This deployment took place in April 2021. The experimental period totalled a PMI of 98 days and 1508ADD at termination (Figure 4.3A). Trial 1 experienced a minimum temperature of 2°C, a maximum temperature of 27°C, and an average daily temperature of 15.4°C. The Trial also endured a total measure of 22.2 mm of rainfall during the trial period (Table 4.1). The initial rate of decomposition for CP1 was accelerated, progressing from a TBS of 3 to 17 in 11 days PMI (207ADD). The EP1 reached a TBS of 17 in 17 days PMI (371ADD), and EP2 reached a similar TBS in 17 days PMI (332ADD). Therefore, CP1 progressed through the initial fresh and early decomposition stages six days faster than the EP1 and EP2, the experimental carcasses progressed with similar rates. The overall progression of pre-rehydration was linear in progression before the carcasses stalled in their decomposition and respective TBS as noted in Figure 4.3A, with the data points highly clustered and similar in their advancement in 17 days PMI (0 to 300ADD). Rehydration took place when the experimental pigs (EP1 and EP2) stalled in their decomposition on PMI day 34 and 32 respectively at a TBS of 20 (617 and 579ADD) (Figure 4.3A).

The progression of decomposition observed post-rehydration for EP1 showed an increase from a TBS of 20 to 23 within 5 days (617 to 683ADD), with a second increase to a TBS of 24,

around 59 days PMI (970ADD), where it remained until 98 days PMI. The EP2 displayed no change and then an increase in decay rate after 20 days post-rehydration from a TBS of 20 to 21, PMI day 50 (579 to 818ADD), with continuous increases occurring until a final TBS of 26 was reached around 96 days PMI (1500ADD). It was noted that EP1 on the next visit showed an increase in the rate of decomposition post-rehydration, that then the TBS tapered, while EP2 had a delayed and more gradual increased decomposition rate but ultimately resulted in a higher TBS before becoming stagnant once more. CP1 progressed through the decay cycle and then stalled at TBS of 22, PMI day 22 to 57 (409ADD until 930ADD) after which there was a small increase to a TBS of 24 around PMI day 62 (1002ADD) after which, decomposition remained unchanged. The average daily temperature throughout Trial 1 displayed a progressive decline starting at ±20°C and ending with ±10°C as the trial transitioned from Autumn into Winter (Figure 4.3B).

Trial 1 experienced five natural rehydration events during the experimental phase, of which, three rainfall events occurred before PMI day 22 (400ADD). In order of occurrences, 1.4 mm of rain fell around PMI day 4 (80ADD), 18.1 mm around PMI day 10 (200ADD), and 1.2 mm around PMI day 18 (330ADD) (Figure 4.3B). Further rainfall occurred around PMI day 42 and 45 (740ADD and 775ADD) with 0.4 mm and 1.1 mm of rain respectively. Although natural rehydration occurred, the carcasses where not directly hydrated, but rather the tarp covered cages and the soil surrounding the carcasses was moistened (indirect natural rehydration). The rain fall occurring at PMI day 4 (80ADD) and PMI day 10 (200ADD) appeared to have no direct effect on the carcasses rate of decay, as they were all still in the fresh stage of decomposition. CP1 experienced a small increase in TBS from 21 to 22 around PMI day 18 (330ADD) which corresponds with the small amount of natural rehydration (Figures 4.3A & B). After the initial increase (TBS of 21 to 22) following natural rehydration. An increase was observed for CP1 (from TBS of 22 to 24) and EP1 (from a TBS f 23 to 24) around PMI day 55 (900ADD), but it was unlikely linked to the natural rehydration event at PMI day 42 and 45 (740 to 775ADD). However, EP2 had an increased in TBS from 20 to 21 shortly after the indirect natural rehydration event at 775ADD (Figure 4.3A & B).

The initial onset of insect activity was seen to be delayed for the whole sample group of Trial 1, with the first noted insect presence around PMI day 4 (±100ADD). EP1 experienced increased insect activity during the early stages of decomposition (PMI day 4 to 38;100 to 700ADD). A similar pattern of insect activity was observed for EP2 with an increase of insect

activity for the early stages of decomposition (PMI day 15 to 27; 300 to 500ADD). The insects were not deterred by the large amount of rain that fell (18.6 mm) around PMI day 10 (200ADD). However, CP1 did show a cease in insect activity shortly after the rainfall event (300ADD), and then insect activity was re-initiated (PMI day 25; 465ADD). The insect activity observed post-rehydration differed between the different pig carcasses. Post-rehydration, EP1 displayed an increase and then a sudden drop to no insect activity around PMI day 42 (740ADD). During the same period, rainfall and a dip in the average daily temperature was observed from an average of ±17°C to ±11°C (Figure 4.3B). Immediately after the indirect natural rehydration the insect activity increased around PMI day 47 (800ADD) and average daily temperature rose to ±18°C (Figure 4.3B & C). The entire carcass was colonised by Hide beetles (*Dermestidae maculatus*) which, then reduced to only having insects present on the abdominal region until the experimental phase ended. EP2 experienced a similar increase and then decrease in insect activity post-rehydration from all three regions being inhabited to only one, namely the trunk (Figure 4.3C). Insect activity fluctuated after this period and then settled with insects similarly inhabiting the abdominal region. CP1 experienced fluctuation of insect activity throughout the experimental phase, experiencing an increased in activity after natural rehydration around PMI day 18 (330ADD) and then tapering down until there was no insect activity up to the end of the trial and decomposition was stagnant (Figure 4.3A & C).

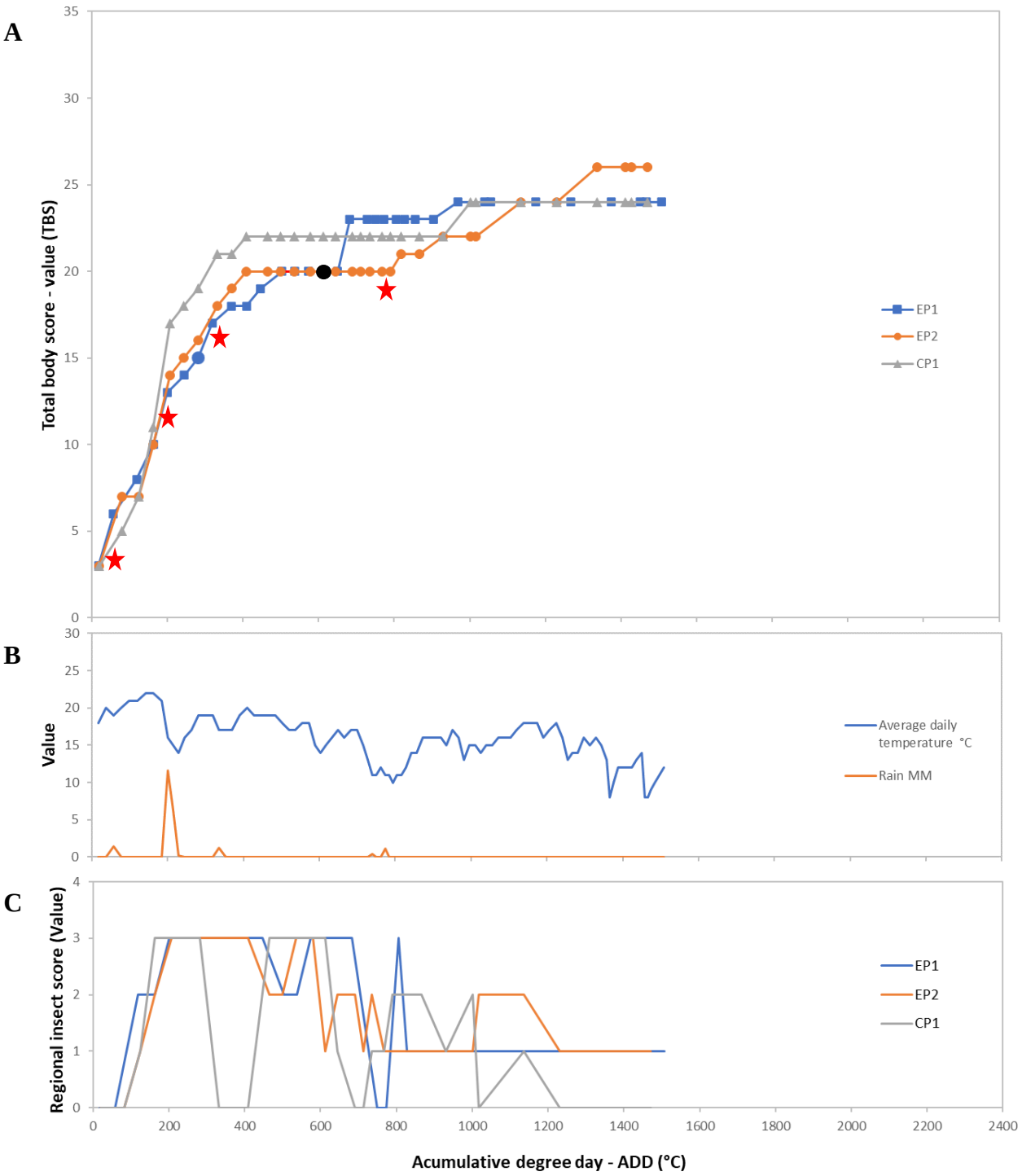

Figure 4.3: **A)** Scatter plot representing accumulative degree days (ADD) against the TBS for the decomposition cycle of Trial 1 with the blue symbol for experimental pig 1 (EP1), orange as experimental pig 2 (EP2), and grey as control pig 1 (CP1). The rehydration event is represented for EP1 and EP2 with a black dot, and indirect rehydration events identified with a star.

B) Average daily temperature and total daily rainfall against ADD, and

C) A graphical representation of the insect activity scored by regional presence of insects (head and neck, trunk, and limbs). A score of 1 representing insects' presence in one region, 2 means presence in two regions and 3 on all regions. The regional scores represent insect activity over the experimental phase plotted against ADD as a time line.

4.2.2 Warmer trials

The warmer deployment took place between July and early August of 2021. For ease of analysis, individual graphs have been made to present the data for each of the warmer trials.

Trial 2

Trial 2 consisted of control pig 2 (CP2) and experimental pigs 3 and 4 (EP3 and EP4). Trial 2 was deployed in July totalling 2283ADD and a PMI of 115 days at termination (Table 4.3 & Figure 4.4). Trial 2 experienced a minimum temperature of 2°C, a maximum temperature of 36°C, and an average daily temperature of 19.9°C. The Trial also underwent a total measure of 23.4 mm of rainfall during the trial period (Table 4.1). The pre-rehydration period in Trial 2 exhibited a more gradual increase followed by a stagnant period experienced by both experimental carcasses (Figure 4.4A). EP3 and EP4 reached a TBS of 21 and 20 around PMI day 73 and 52 (1350ADD and 885ADD), respectively. CP2 reached a stagnant period at a TBS of 29 at around PMI day 93 (1750ADD). The initial rate of decay was slowed for all the carcasses, with the data points of related TBS more scattered distribution. The CP2 and EP4 followed a more similar rate and progression of decomposition, whereas EP3 progressed through the initial fresh and early stage of decomposition over a far greater time frame.

The progression observed post-rehydration for EP3 displayed the carcass remaining with a TBS of 22 for 25 days before increasing to a TBS of 24 and becoming stagnant once more (PMI day 83 and 108; 1540 to 2115ADD respectively). While EP3 remained stagnant for most of the time after rehydration, EP4 did not. The EP3 carcass was not terminated after three visits of the TBS score not changing post-rehydration due to the active decomposition of both the CP2 and EP4 carcasses. The decomposition of EP4 stalled at a TBS of 20 and appeared to increase immediately after rehydration to TBS of 24 in 3 days (PMI day 63 to 66; 1110 to 1170ADD) and then increased gradually over the next 17 days to a TBS of 27 (1166 to 1540ADD) and to a TBS of 30 over the next 22 days (1962ADD), when decomposition became stagnant again (Figure 4.4A). CP2 followed a pattern of gradual increase in TBS with staggered stalling episodes of two visits respectively (TBS of 6, 10, 14, 17, 18, & 22 etc.). After every re-initiation in TBS the carcass eventually entered a stagnant period occurring at TBS of 30 around PMI day 94 (1790ADD). A slight increase of TBS occurred for CP2 around PMI day 107 (2110ADD) until the end of the trial phase. It was therefore noted, that the EP4 displayed a

direct response to artificial rehydration and EP3 displayed no direct effect to the artificial rehydration event.

Indirect natural rehydration occurred during the experimental phase of Trial 2 with a decrease in average daily temperature during the rainfall periods. Rainfall occurred in seven distinct events, some expanding over a few days. All the rainfall events only occurred after 590ADD, with the events taking place with 0.9 mm of rainfall around PMI day 37 (590ADD), 1.7 mm around PMI day 50 (840ADD), 2.9 mm between PMI day 67 and 74 (1200 and 1350), 6.3 mm between PMI day 76 and 80 (1400 and 1480ADD), 2.4 mm around PMI day 90 (1700ADD), 1.7 mm between PMI day 94 and 104 (1800 and 2000ADD), and 5.2 mm between PMI day 108 and 115 (2100 and 2300ADD) (Figure 4.4B).

CP2 experienced a stalled decomposition rate for two visits (PMI day 37, 50, and 80) and then increased in TBS (TBS of 14, 28, and 30 at 590, 840, 1480ADD respectively). The control carcass (CP2) displayed changes in rate of decay after indirect natural rehydration around PMI day 37 (590ADD) with a re-initiation in TBS observed from a score of 14 to 17 in 2 days (612 to 660ADD). Another increase in TBS was observed for CP2 and EP4 at the same time as an indirect natural rehydration event (840ADD) resulting in a change of TBS of 18 to 20 (890 to 960ADD) and 17 to 21 (830 to 890ADD). As mentioned, around PMI day 37 an indirect natural rehydration event was linked to an increase in the decomposition of EP3 from a TBS of 9 to a score of 11 and EP4 increasing from a TBS of 12 to 15 around PMI day 38 (600ADD). A similar change in the rate of decomposition was observed at approximately 1350, 1460, and 1700ADD respectively for EP4. However, continuous showers around PMI day 80 to 115 (1480 to 2300ADD) were observed towards the end of the trial, with no change observed in the rate of decay for EP3 except a final increase in TBS from 22 to 24 around PMI day 105 to 108 (2040 to 2100ADD respectively) before termination of the trial. A change in progression of decay was observed for EP4 with a jump from a TBS of 28 to 30 around PMI day 98 to 102 (1880 to 1960ADD), the change occurred during the continuous showers occurring between PMI day 80 and 115 (1480ADD and 2300ADD). The EP4 carcass did experience indirect natural rehydration at a TBS of 27 and remained unchanged for 8 days (1540 to 1720ADD) before increasing gradually over the following 8 days to 30 TBS (1720 to 1960ADD), after which, decomposition became stagnant once more (Figure 4.4A & B). The rate of decay is seen to be altered for almost every indirect natural rehydration event for all the sample group pigs respectively, with exception to EP3 during the advanced stage of decomposition.

The onset of insect colonisation was not consistent for all carcasses, insect activity was observed on EP3 from day 2 PMI (17ADD). Whereas, both CP2 and EP4 had an appeared delay in insect colonisation, only occurring around four days PMI (110ADD). The insect activity at the beginning of the trial fluctuates for all the carcasses during the initial early decomposition stage, simultaneously the insect activity appeared less during cooler temperatures around PMI day 25, 40, and 90 (370, 650, and 1720ADD) (Figure 4.4C). A qualitative analysis of the insect activity in Trial 2 revealed reduced fly activity and maggot migration with small maggot masses in the mouth, anus and a few observed under the carcass in the purged decomposition fluids. Only during the advanced stage of decay did Hide beetles (*Dermestidae maculatus*) appear to colonise and begin to consume the desiccated flesh for all the Trial 2 carcasses. For EP3 insects appeared active over the entire carcass prior to rehydration, consisting of mainly Hide beetles. Post-rehydration, insect activity continued over all regions of the carcass until the end of the experimental period (Figure 4.4C). The EP4 experienced an increase in insect activity after rehydration, increasing from activity in two regions to three. The activity mainly consisted of Hide beetles and flies but no new larvae were visually present. EP4 experienced two indirect natural rehydration after artificial rehydration and the pattern of insect activity appeared to decrease after the first indirect natural rehydration event around PMI day 83 (1540ADD), and an increase in insect activity after a second smaller amount indirect natural rehydration (PMI day 102; 1960ADD). The increase in insect activity occurred simultaneously with an increase in the TBS around PMI day 94 (±1800ADD). CP1 appeared to experienced fluctuating insect activity in early decomposition stages with increased activity during the middle of the decay process which then tapered down with a slight increase observed after a small amount of indirect natural rehydration around PMI day 79 (1450ADD), followed by complete cessation until the end of the experimental phase. It was therefore observed that both direct and indirect rehydration alter insect activity in deterring activity and causing an increase in activity.

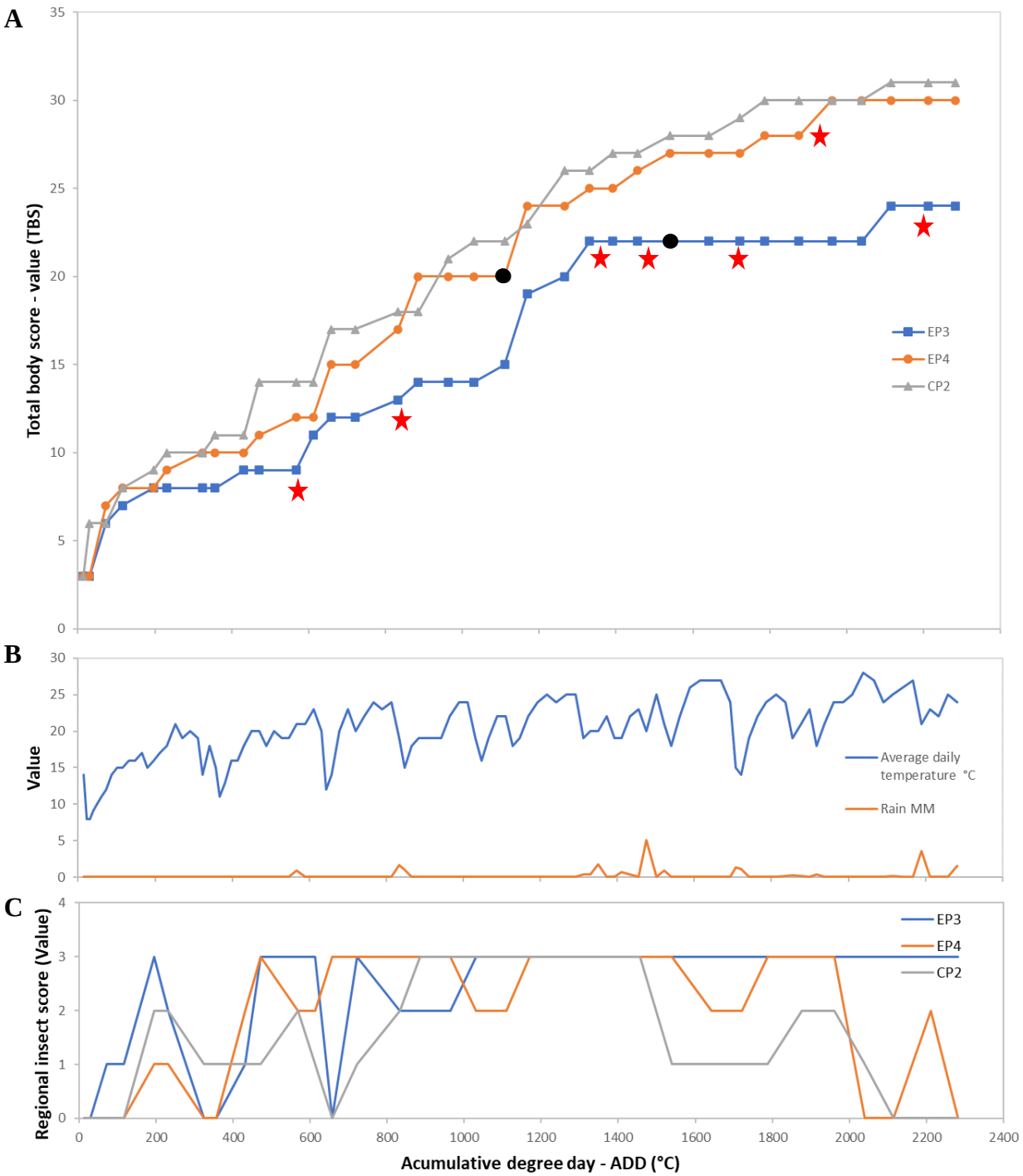

Figure 4.4: **A)** Scatter plot representing accumulative degree days (ADD) against the TBS for the decomposition cycle of Trial 2 with the blue symbol for experimental pig 3 (EP3), orange as experimental pig 4 (EP4), and a grey as control pig 2 (CP2). The rehydration event is represented for EP3 and EP4 with a black dot, and indirect rehydration events identified with a star.
B) Average daily temperature and total daily rainfall against ADD, and
C) A graphical representation of the insect activity scored by regional presence of insects (head and neck, trunk, and limbs). A score of 1 representing insects' presence in one region, 2 means presence in two regions, and 3 on all regions. The regional scores represent insect activity over the experimental phase plotted against ADD as a time line.

Trial 3

Trial 3 was deployed shortly after Trial 2 in early August 2021 and totalled an ADD of 1941 in 92 days PMI at termination (Table 4.3). Trial 3 experienced a minimum temperature of 4°C, a maximum 36°C and an average daily temperature of 21.1°C (Table 4.1). The Trial also underwent a total measure of 23.4 mm of rainfall during the trial period (Table 4.1). The carcasses reached a point of stasis each at different points in time. CP3 reached a TBS of 32 in 68 days PMI (1380ADD), EP5 reached a TBS of 22 in 40 days PMI (768ADD), and EP6 had a TBS of 25 at 55 days PMI (1127ADD) (Figure 4.5). The EP5 carcass experienced a greater initial rate of decomposition when compared to CP3 and EP6. Although CP3 and EP6 progressed at a slower rate, the TBS for both carcasses increased in a more uniform manner with gradual increases before reaching an initial plateau in early phase of decomposition around PMI day 36 and 25 (622 and 470 ADD respectively). Only once the EP6 carcass stalled in advanced decomposition was it rehydrated (PMI day 50; 980ADD). The progression of pre-rehydration decomposition followed a sigmoidal distribution with data points of TBS clustered and displaying an initial rapid increase in TBS and a plateau phase where artificial rehydration took place for the experimental carcasses.

Post-rehydration decomposition for EP5 displayed an increase from a TBS of 22 to a TBS of 24 in 7 days from 768ADD to 925ADD (Figure 4.5A) with a second stagnant phase occurring at TBS of 25 around PMI day 56 (1114ADD). Post-rehydration progression of decomposition for EP6 displayed a TBS moving from 25 to 27 (1127 to1309ADD) in 8 days followed by a second and third stagnant phase at TBS 26 and 27 around PMI day 66 and 80 (1374 and 1702ADD), respectively. Both EP5 and EP6 displayed a gradual increase in decay, post-rehydration. The decomposition of CP3 followed a rapid decomposition rate until stalling slightly at a TBS of 17 for 7 days (622 to 768ADD) before reaching a stalled TBS of 32 around PMI day 68 (1380ADD) when the decomposition enters a state of stasis (Figure 4.5A). Both experimental carcasses had an increase in the rate of decay post-rehydration and the increase occurring ±9 days.

Natural rehydration occurred numerous times throughout the experimental phase of Trial 3, with approx. seven rainfall events like the rain that fell during Trial 2. However, the first rainfall event occurred at 230ADD during the early stage of decomposition with the other events taking place with 0.9 mm of rainfall around PMI day 13 (230ADD), 1.7 mm around PMI day 27 (500ADD), 2.9 mm between around PMI day 51 (1000 and 1070), 6.3 mm between PMI day

55 and 60 (1100 and 1200ADD), 2.4 mm around PMI day 67 (1360ADD), 1.7 mm between PMI day 73 and 84 (1500 and 1750ADD), and 5.2 mm between PMI day 86 and 90 (1800 and 1900ADD) (Figure 4.4B). The rainfall indirectly rehydrated the carcasses by landing on the tarp covered cages and the ground surrounding the carcass moistening the soil where the carcasses lay. The EP5 had stalled at 22 TBS (500ADD). However, EP5 did not display a change in the decomposition as the carcass remained stalled in decomposition and then was artificially rehydrated at a TBS of 22, PMI day 40 (768ADD), represented by the black dot in Figure 4.5A. There was a re-initiation seen in the TBS after another event of indirect natural rehydration for EP5 (TBS of 25 at 1360ADD), after which, the carcass progressed to a higher TBS than EP6 before becoming stagnant once more. Pre-rehydration, at 500ADD, rain fell and an increase in decomposition rate was observed for EP6. The EP6 carcass experienced indirect natural rehydration shortly after the artificial rehydration (Figure 4.5A & B), following which, the decay slowed to a stall. A small amount of constant indirect natural rehydration was observed between PMI day 67 and 84 (1360 – 1750ADD). During this period, EP5 experienced a gradual increase in decay going from a TBS of 25 to 30 in 19 days (1380 to 1690ADD). EP6, that had become stagnant at a TBS of 27 (1374 to 1626ADD), then had a slight increase in decomposition, increasing to a TBS of 28 (PMI day 82; 1700ADD) (Figure 4.5A & B). CP3 displayed a stalled period of decomposition at a TBS of 17 (PMI day 33; 622ADD) which coincided with a natural rehydration event occurring around the same time, after which CP3 exhibited an increase in decomposition rate.

The onset of insect activity was staggered for the three carcasses in Trial 3 with EP5 experiencing an immediate onset of insect activity, EP6 at PMI day 6 (90ADD), and CP3 at PMI day 8 (130ADD) (Figure 4.5C). The initial fly activity and maggot colonisation occurred and as the carcasses dried out the beetle populations colonised the carcass and consumed the dried-out skin until the carcasses were skeletonised. The EP5 experienced a cease in insect activity in the early phase of decomposition that occurred after a major decrease in the average daily temperature and coincided with a natural rehydration event at 500ADD (Figure 4.5B & C).

The insect activity observed over the period of Trial 3 indicated a decrease in insect activity for EP5, EP6, and CP3 from PMI day 42 to 46 (800 to 900ADD). During this period, no rainfall or a major drop in average daily temperatures were observed. Post-rehydration, EP5 experienced a sudden cease in the insect activity and then a re-initiation was observed,

occurring together with an increase in TBS (TBS of 22 to 24) around PMI day 40 (770ADD). However, as the decomposition cycle progressed the insect activity decreased after an indirect rehydration event (PMI day 95 to 64; 925 to 1300ADD) and then stalled with insects only visible on the abdominal region (Figure 4.5B & C). The EP6 experienced great fluctuation in insect activity throughout the experimental phase.

Prior to rehydration, insects were present on the carcass in two regions namely the trunk, as well as the head and neck around PMI day 22 to 26 (400- 500ADD), however once artificially rehydrated (1127ADD) the insect activity decreased to only being present on the abdomen and then re-initiation on all three regions being colonised by Hide beetles (*Dermestidae maculatus*) (1460ADD) (Figure 4.5C). A small amount of rain (±1.4 mm) fell around PMI day 78 (1600ADD) and a cease in insect activity was observed with a re-initiation resulting in complete insect colonisation of EP6 (Figure 4.5B & C). CP3 experienced extensive insect activity during the early to advanced decomposition phase. CP3 displayed a dip in insect activity around PMI day 47 (925ADD) with an increase and subsequent decline in insect activity around PMI day 51 and 64 (1000ADD and 1300ADD), respectively, which coincided with a cease in decomposition (Figure 4.5A and C). It was therefore noted that insect activity was initially deterred after rehydration took place and then increased as time passed, possibly once the carcass had dried out becoming more suitable for hide beetles as their presence made up the main invertebrate colonisers post-rehydration.

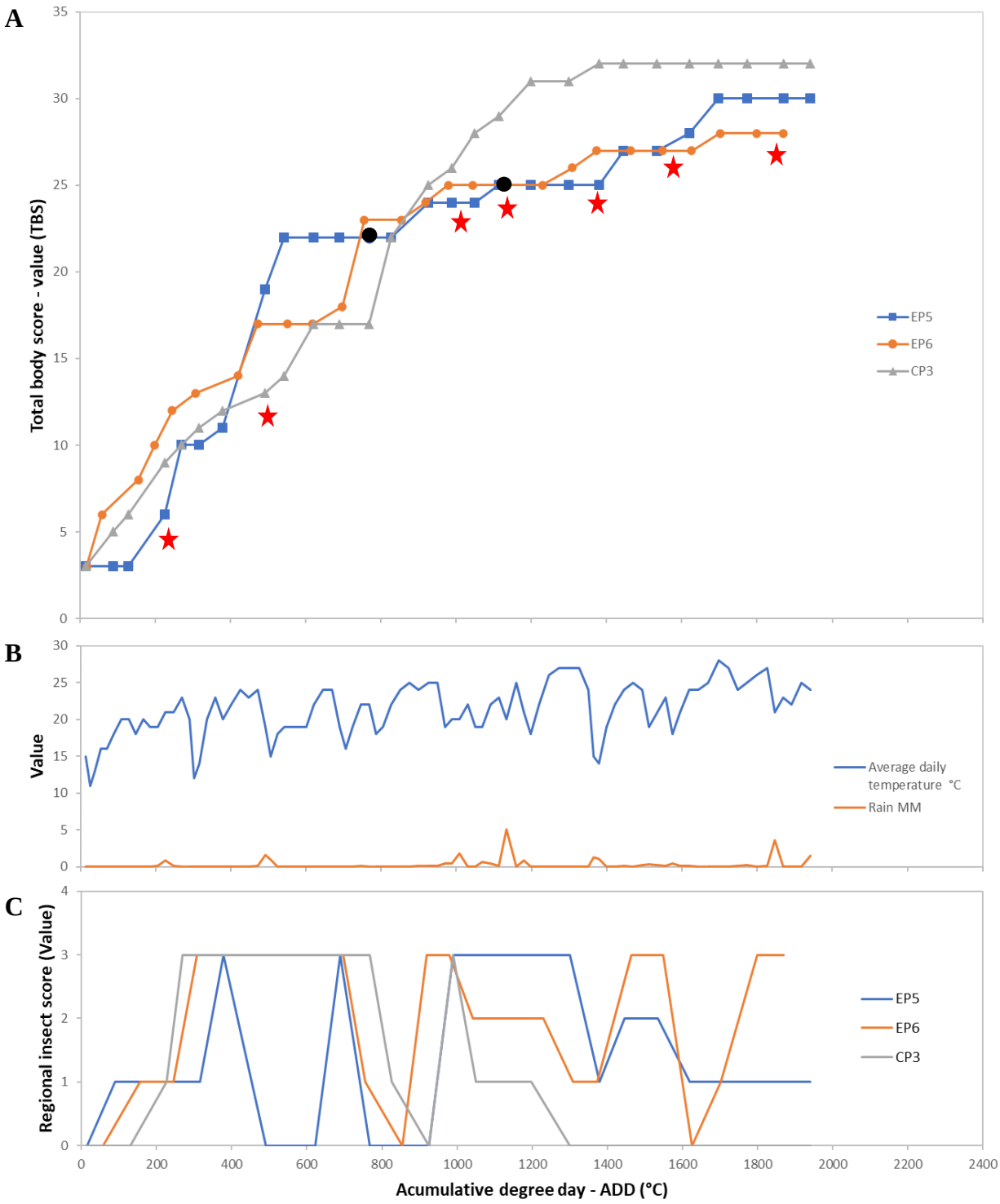

Figure 4.5: **A)** Scatter plot representing accumulative degree days (ADD) against the TBS for the decomposition cycle of Trial 3 with the blue symbol for experimental pig 5 (EP5), orange as experimental pig 6 (EP6), and a grey as control pig 3 (CP3). The rehydration event is represented for EP5 and EP5 with a black dot, and indirect rehydration events identified with a star. **B)** Average daily temperature and total daily rainfall against ADD, and
C) A graphical representation of the insect activity scored by regional presence of insects (head and neck, trunk, and limbs). A score of 1 representing insects' presence in one region, 2 means presence in two regions, and 3 on all regions. The regional scores represent insect activity over the experimental phase plotted against ADD as a time line.

Trial 4

Trial 4 was deployed in August and early September totalling 1625ADD at termination and extended over a period of 74 days post-mortem (Table 4.3 and Figure 4.8). Trial 4 experienced a minimum temperature of 10°C, a maximum 36°C and an average daily temperature of 21.9°C (Table 4.1). The Trial also underwent a total measure of 22.3 mm of rainfall during the trial period (Table 4.1). CP4 reached a TBS of 19 around PMI 23 (468ADD). EP7 displayed a similar TBS of 21 at PMI 32 (452ADD), and EP8 reached a TBS of 19 at PMI 32 (511ADD). Although the TBS values occurred over a short period of time, for the initial stages of decomposition the changes in TBS increased rapidly. The progression of decomposition pre-rehydration for Trial 4 displayed a sigmoidal distribution with the linear early stage of decomposition and stalling at the plateau phase and the carcasses were rehydrated, then re-initiating decomposition to the point of skeletonisation.

Rehydration for Trial 4 took place different to that of Trial 1, 2, and 3. The carcasses of Trial 4 were placed within the secondary cages (Figure 4.2), however due to excessive wind the tarps protecting the carcasses from direct natural rehydration lifted and the two experimental carcasses where fully rehydrated with approximately 2.8 mm of rain as recorded by World weather online (2022) (Figure 4.5, 4.6, 4.8A & B). However, due to the placement of the tarp it is thought that the water was concentrated onto the carcass and may have exposed the carcass to more rain than recorded. The skin of both carcasses notably changed colour post-rehydration, but the colour change did not persist (Figure 4.5 & 4.6). The skin changed from brown to near white in colour, however the colour returned to brown and dried out on the next visit (734ADD). Both EP7 and EP8 had stalled in advanced decomposition before the natural rehydration took place (PMI day 32; 673ADD), which was in line with the artificial rehydration protocol of the study. Post-rehydration for both EP7 and EP8 had a re-initiation in TBS. The EP7 showed an increase from a TBS of 21 to 25 in 6 days (673 to 800ADD) (Figure 4.8A). The EP8 experienced an increase in decay from a TBS of 19 to 22 in 3 days (673 to 734ADD) after which it remained there for 29 days (734 to 1300ADD) before a slight increase to a TBS of 24 (PMI day 64; 1381ADD) where the carcass remained stagnant. It was therefore noted, that rehydration of the experimental carcasses was linked with an increase in the rate of decomposition resulting in an increase TBS.

Indirect natural rehydration was noted to take place in seven distinctive events like that of Trial 2 and 3. The initial rainfall occurred was observed around PMI day 10 (±200ADD) with 1.8

mm of rain, a rapid increase in TBS (TBS of 6 to ±10) was observed for all the carcasses. Rain fell between PMI day 36 and 38 (750 and 800ADD) totalling 6.3 mm, however, neither EP7 or EP8 had an increase in the rate of decay. CP4 did subsequently have an increase in the rate of decomposition progressing from a natural rehydration event occurred at around PMI day 44 (940ADD) (2.4 mm of rain) with changes in the rate of decomposition only observed for CP4 and EP7 with a TBS of 23 and 25 increasing to 25 and 26 respectively. After the continuous showers observed around PMI day 52 to 61 (1100 to 1300ADD) a change of TBS from 22 to 24 was recorded for EP8 (1300ADD). CP4 was exposed to the indirect natural rehydration observed at PMI day 32 (673ADD), however the tarp served to stop the control from being completely exposed to direct natural rehydration (Figure 4.8A & B). CP4 had become stagnant around the same time as the experimental pigs (EP7 and EP8), however after the indirect rehydration event the TBS increased from 19 to 20 in 3 days (630 to 691ADD). EP7 progressed from a TBS of 25, ending at a higher TBS of 30 around PMI day 67 (1475ADD), whereas CP4 was at a TBS of 23 at PMI day 44 (941ADD) and stalled in decay at a TBS of 25 around PMI day 47 (1000ADD) up to the end of the experimental phase (Figure 4.8A & B). Indirect natural rehydration appeared to only effect the rate of decomposition for EP7 and CP4, while artificial rehydration increased the rate of decomposition of EP8 initially after which the carcass stalled at a TBS of 22 (734ADD), followed by a slight increase around PMI day 63 (±1350ADD).

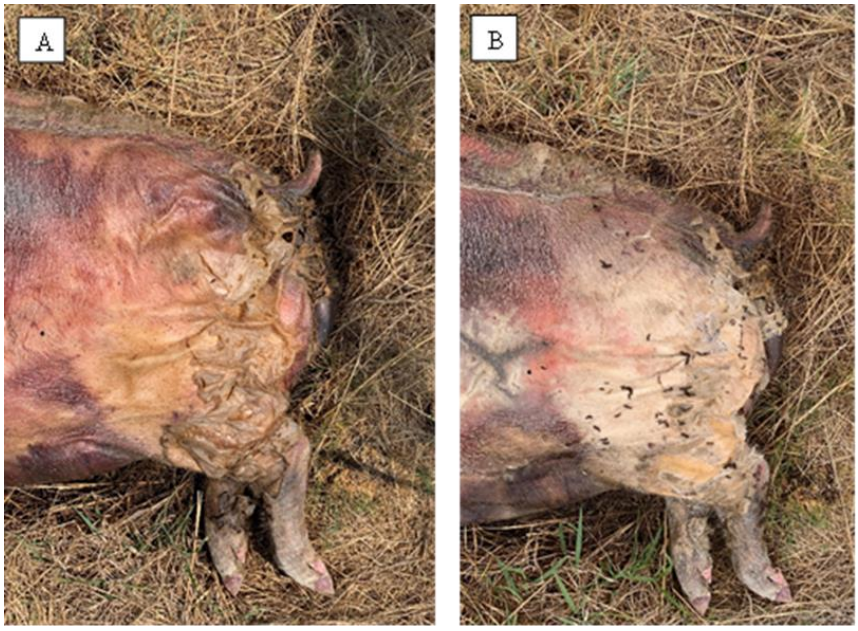

Figure 4.6: Hind limbs of experimental pig 7 (EP7) from Trial 4 illustrating skin discoloration post-rehydration. **A)** EP7 on PMI 29 (609ADD) with appearance of brown dried out tissue, and **B)** EP7 on PMI 32 (673ADD) post natural direct rehydration with white water saturated tissue and increased insect activity visible.

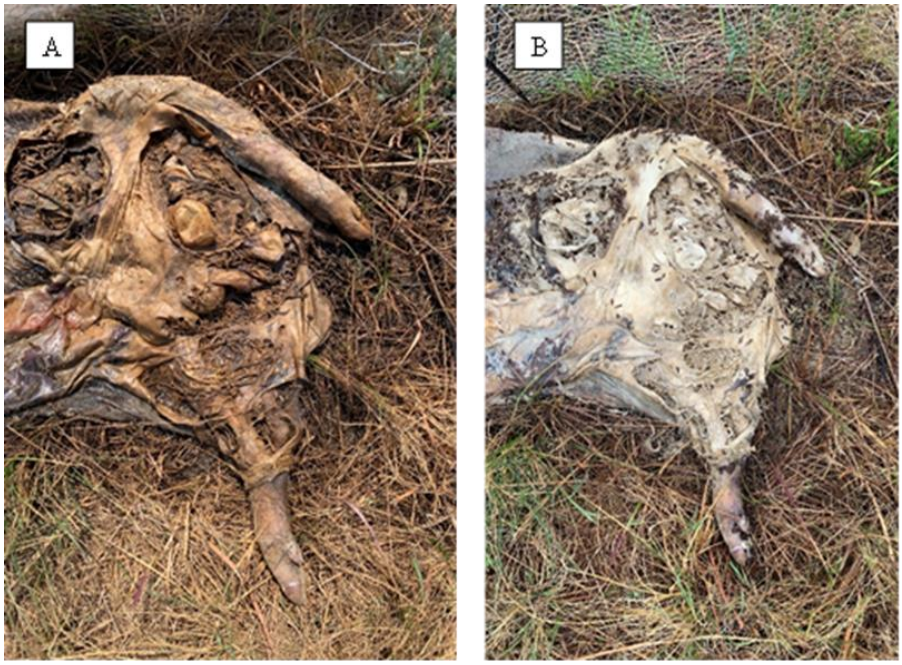

Figure 4.7: Hind limbs of experimental pig 8 (EP8) from Trial 4 illustrating skin discoloration post-rehydration **A)** EP8 on PMI 29 (609ADD) with appearance of brown dried out tissue, and **B)** EP8 on PMI 32 (673ADD) post natural direct rehydration with white water saturated tissue and increased insect activity visible.

Post-rehydration EP7 exhibited an increase in insect activity from no insect activity (PMI day 29; 600ADD) to insects on all regions of the body (PMI day 32;680ADD). However, a large amount of indirect natural rehydration occurred around PMI day 39 (±818ADD) resulting in decreased insect activity (Figure 4.8B & C). As decomposition progressed, the insect activity declined until insects were only present on the abdominal region until the end of the experimental phase (Figure 4.8A & C). Post-rehydration, EP8 experienced a more gradual increase in insect activity, with Hide beetles and Diptera present but no visible fly larvae were noted. The insect activity was decreased after continuous rainfall seen around PMI day 56 to 65 (1200 – 1400ADD) and then increased again with insects active on all regions of the carcass until the end of the experimental phase. CP4 experienced an initial increase of insect activity during the early phase of decomposition which stalled around PMI day 26 (±550ADD) where CP4 also stalled in decay. However, indirect rehydration event occurred (PMI day 40; ±800ADD) and insect activity and decay were re-initiated although only one region incurred insect activity until around PMI day 57 (±1200ADD) after the carcass had stalled in decay and insect activity had ceased (Figure 4.8A & C).

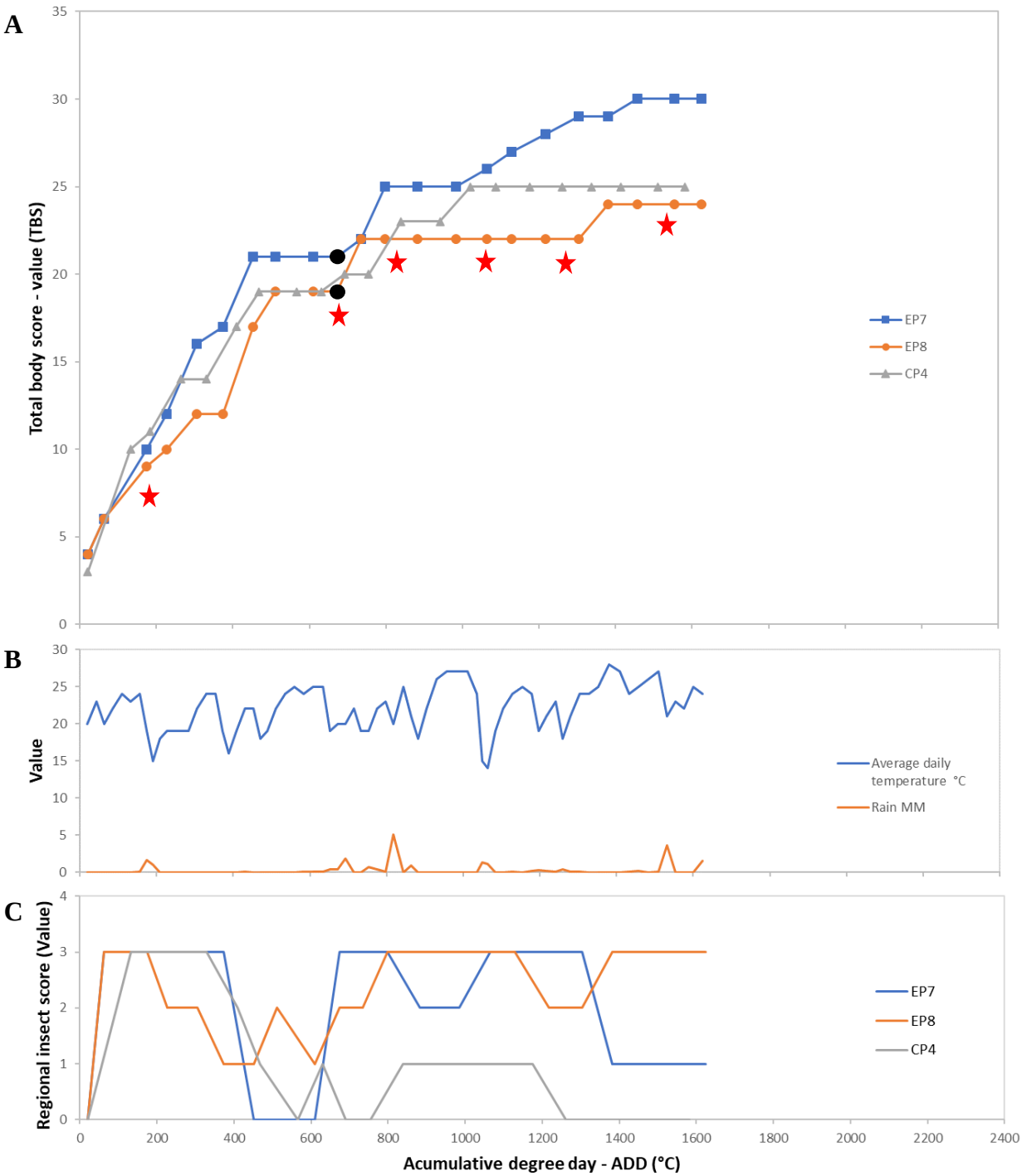

Figure 4.8: **A)** Scatter plot representing accumulative degree days (ADD) against the TBS for the decomposition cycle of Trial 4 with the blue symbol for experimental pig 7 (EP7), orange as experimental pig 8 (EP8), and a grey as control pig 4 (CP4). The rehydration event is represented for EP7 and EP8 with a black dot, and indirect rehydration events identified with a star.
B) Average daily temperature and total daily rainfall against ADD, and
C) A graphical representation of the insect activity scored by regional presence of insects (head and neck, trunk, and limbs). A score of 1 representing insects' presence in one region, 2 means presence in two regions, and 3 on all regions. The regional scores represent insect activity over the experimental phase plotted against ADD as a time line.

4.2.3 Comparisons of decomposition

The results of the four trials were categorized based on the time of deployment with differing weather conditions, resulting in a cooler and three warmer trials. The pigs were further categorized into experimental and control pigs, where the experimental pigs experienced artificial rehydration by the researcher. A comparison of the decomposition was be done to summarize the findings of all four trials, focusing on general rate and progression of decomposition between cooler and warmer trials, temperature, pre- and post-rehydration decay rates, indirect rehydration, and general observations.

Temperature

As mentioned, the four trials were deployed at different points of the year and were subsequently exposed to differing climatic conditions. Table 4.1 details the minimum, maximum, and average daily temperatures for all four trials respectively. Trial 1 endured the lowest maximum temperature (27°C) and average daily temperature (15.4°C), with an average daily temperature ±5°C lower than Trial 2, 3, and 4 as noted in table 4.2.

Within the first seven days of deployment each trial experienced an average temperature of approximately 20°C for Trial 1, 10 °C for Trial 2, 15.5°C for Trial 3, and 22°C for Trial 4. The average daily temperature was seen to drop when natural rehydration occurred (Figure 4.3, 4.4, 4.5 & 4.8). The stalling of decomposition was noted when there was a decrease in temperature, for example in Trial 1 at ±700ADD (Figure 4.3A & B). The last set of pigs deployed for Trial 4 (represented by red) experienced the highest average temperature and displayed a faster rate of decomposition when compared to Trials 2 and 3. Trial 4 had carcasses end with similar final TBS scores to Trial 1 but ended with a greater ADD value.

Pre-rehydration

The rate of decomposition was compared for the individual trials by calculation of the number of days the carcasses took to reach a TBS of 17 and the related ADD values. A TBS of 17 was chosen as it represents the initial stages of decay (fresh and early stage of decomposition) (Finaughty & Morris, 2019), before the carcasses enter the advanced stage of decomposition and active decay has stalled and where the carcass begins to dry out. The initial rate of decomposition between the trials revealed that the average PMI taken by Trial 1 to reach a TBS of 17 was ±15 days PMI and ±303ADD; the average PMI taken by Trial 2 to reach TBS

of 17 was ±52 days PMI and ±886ADD; the average PMI taken by Trial 3 to reach TBS of 17 was ±27 days PMI and ±577ADD; and the average PMI taken by Trial 4 carcasses to reach TBS of 17 was ±20 days PMI and ±425ADD. Therefore, the cooler Trial 1, on average progressed through early to advanced decomposition at a greater rate than Trial 2, 3, and 4 (Figure 4.10). When observing the dates when the carcasses were rehydrated, it was noted the cooler trial (Trial 1) was rehydrated a month after deployment and took two months to terminate post-rehydration. As compared to the warmer trials (Trial 2 and 3), it took 2 to 3 months to rehydrate the carcasses although not for all cases. Trial 4 was rehydrated within a month of deployment. All the warmer trials were terminated within one month of rehydration.

Another observation between the trials was the difference in rate of decay of the "larger pigs" as seen from the estimated weights displayed in Table 4.3 for EP6 (±100 kg) in Trial 3 and EP3 (±90 kg) in Trial 2. EP6 took 24 days post-mortem (472ADD) to reach a TBS of 17 and EP3 took 42 days (672ADD) longer to reach TBS of 15 (PMI of 66 days at 1110ADD). The EP3 stalled at TBS of 22 (1331ADD) and EP6 stalled at TBS of 25 (979ADD). Therefore, although climatic conditions and weight were similar, EP3 decayed as a much slower rate through the initial stages of decomposition and stalled at a TBS of 22 compared to the EP6 stalling at a TBS of 25 for a much shorter time.

When comparing the progression and rate of the control pigs (CP1, CP2, CP3, and CP4), it was noted the warmer trial controls had a slower rate of decay but reached a higher end TBS with an average of 29 before decomposition stalled as compared to CP1 with a TBS of 24. The cooler control pig (CP1) experienced a greater increase from fresh to advanced decomposition and then stalled in the advanced phase (seen in Appendix D figure A.1). The CP4 experienced different temperature conditions yet the rate of decay was like that of CP1.

When comparing the progression and rate of decomposition, the trials differed in their distribution slightly. Trial 1 experienced a linear pattern for the initial progression of decay, with a clustered distribution of TBS values. Trial 2 displayed a more gradual distribution and progression of TBS values through the initial decay process. Trial 3 progressed rapidly through the initial stage of decay and Trial 4 also progressed with a rapid linear distribution through the initial stage of decomposition. All the carcasses progressed through decomposition with the limbs skeletonising first followed by the head with prolonged insect activity in the trunk region until skeletonisation.

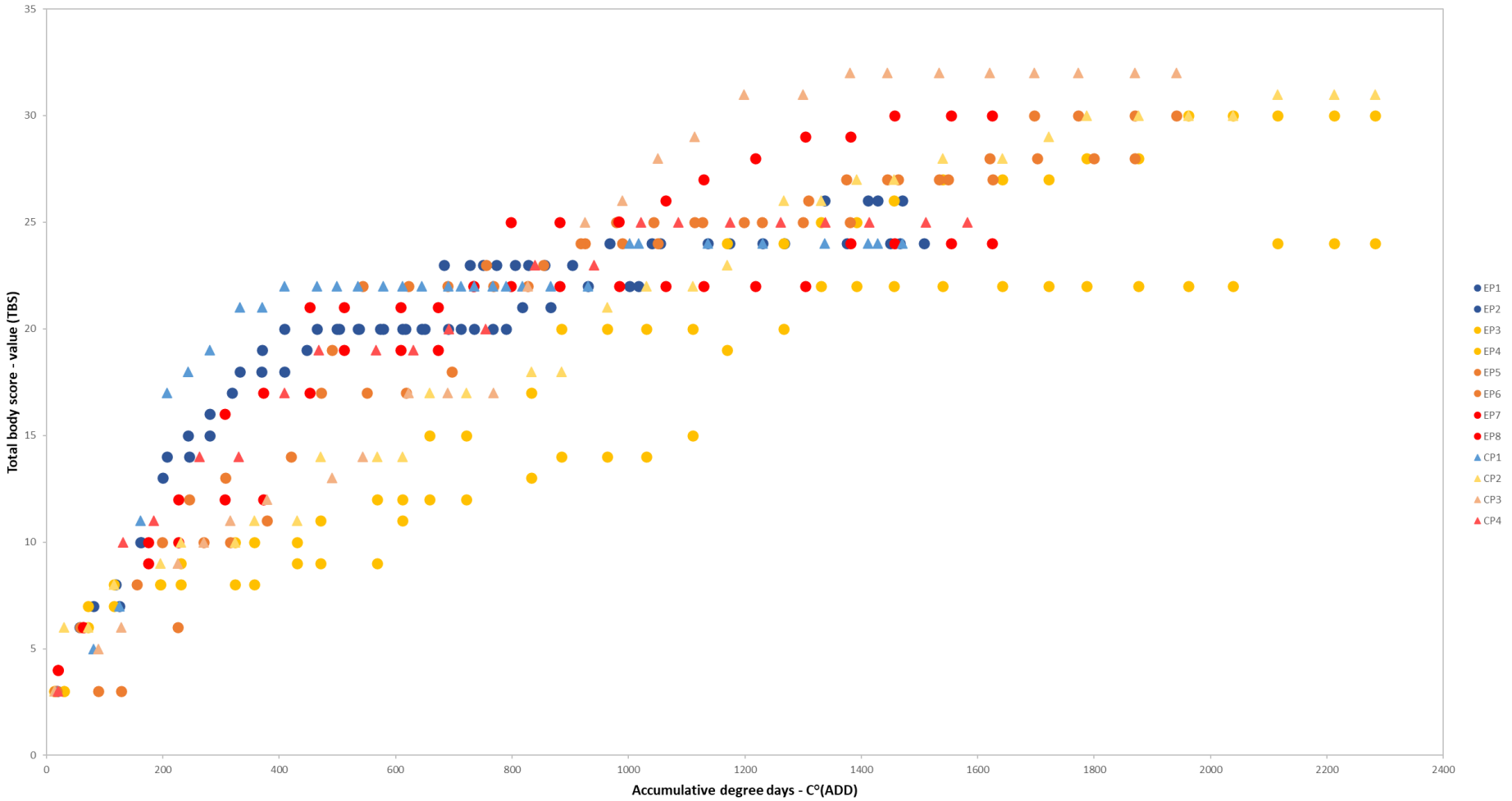

Figure 4.9: Comparison of rate of decomposition between all control (triangles) and all experimental (circles) pigs from all four Trials by plotting TBS against accumulative degree days (ADD). Colours representing colder (blue) to warmer (yellow, orange, and red) weather conditions throughout experimental phase.

Post-rehydration

Rehydration is seen to change the decomposition rate in the experimental carcasses compared to the control carcasses for both the warmer and cooler trials (Figure 4.9 & seen in Appendix E figure A.2). The rehydration event is seen to re-initiate the rate of decay, with the TBS values increasing after a state of stasis. Post-rehydration differed between Trial 2, 3 and 4 compared to Trial 1, where the data points are far more clustered together as opposed to the warmer trials (Figure 4.3A, 4.4A, 4.5A, & 4.8A). The cooler Trial 1, averaged 12.5 days (±135ADD) to observe a change in the visible decay resulting in a change of TBS by an average of 2 TBS points. The warmer trials (Trial 2, 3, and 4) averaged ±9 days (±242ADD) to observe an average change in decomposition by 2.8 TBS points. The two pigs that took the longest to change in TBS, post-rehydration, were EP2 (20 days, ±201ADD, by 1 TBS point), and EP3 (25 days, ±575ADD by 2 TBS points). Both the larger pigs (EP3; EP6) took longer to decompose, increasing their TBS by 2 TBS points over 25 (±182ADD) and 11 days (±575ADD), respectively.

As seen in Figure 4.9, the experimental sample for all four trials displayed a trend of continued decay after rehydration. The control sample in all four trials progressed in its decay uniformly and, once reaching stasis, remained unchanged, or displayed small increases, which corresponded with natural rehydration events until complete desiccation or skeletonisation took place. Furthermore, the cooler trials data points are far more clustered, occur over a much shorter ADD and stalled at a lower TBS (Figure 4.9 – blue data). The warmer trials data is far more gradual and spread-out, taking longer to reach a larger TBS and ADD, and the cooler trials display a faster rate of decomposition (Figure 4.9 – yellow, orange, and red data).

An interesting observation noted during Trials 2 and 3 included the experimental carcasses (EP4 and EP5) displaying a greater cadaver decomposition island (CDI) when compared to the respective control pigs that had not been directly rehydrated (Figure 4.10 & 4.11). A CDI is the visible deposition of broken-down tissue from decomposition, observed as a dark stain surrounding a cadaver/carcass (Fancher et al., 2017). Smaller CDIs were observed for all pigs, however the length of the grass surrounding some of the pigs (EP1 and EP2) made the size of the CDI unclear.

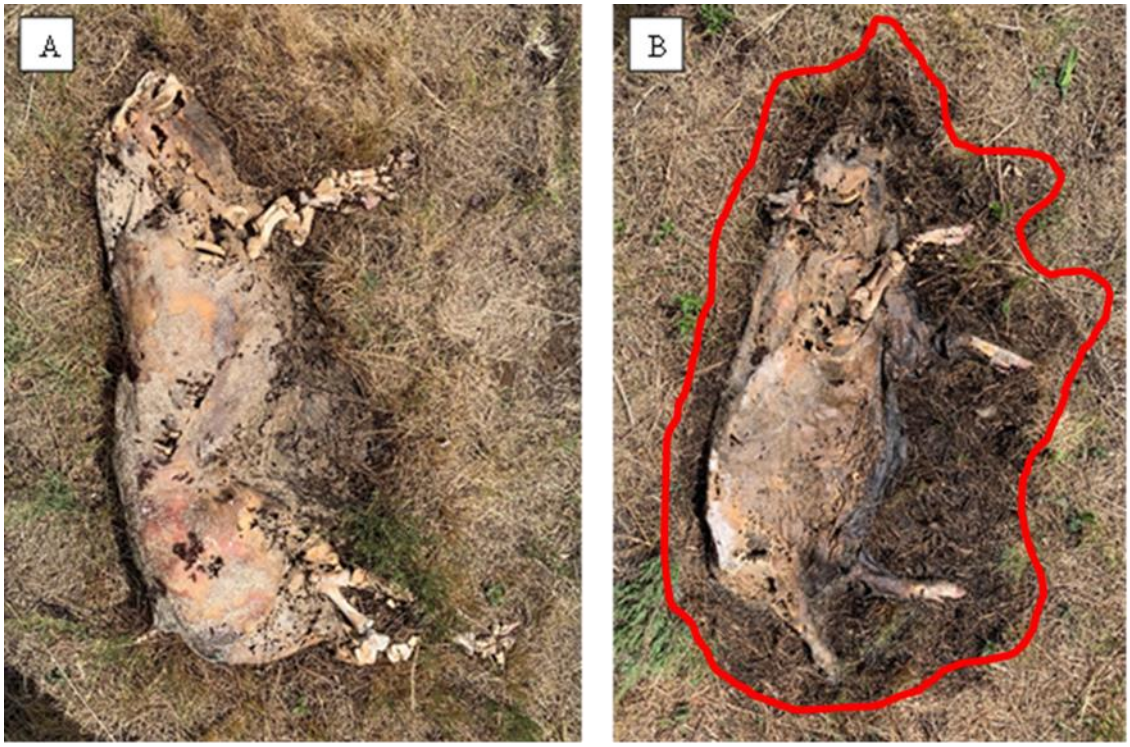

Figure 4.10: **A**) Control pig 2 on PMI 108 days (2115ADD), and **B**) experimental pig 4 on PMI 108 days (2115ADD), post-rehydration of Trial 2 displaying a greater CDI (dark stain on ground surrounding the carcass outlined in red).

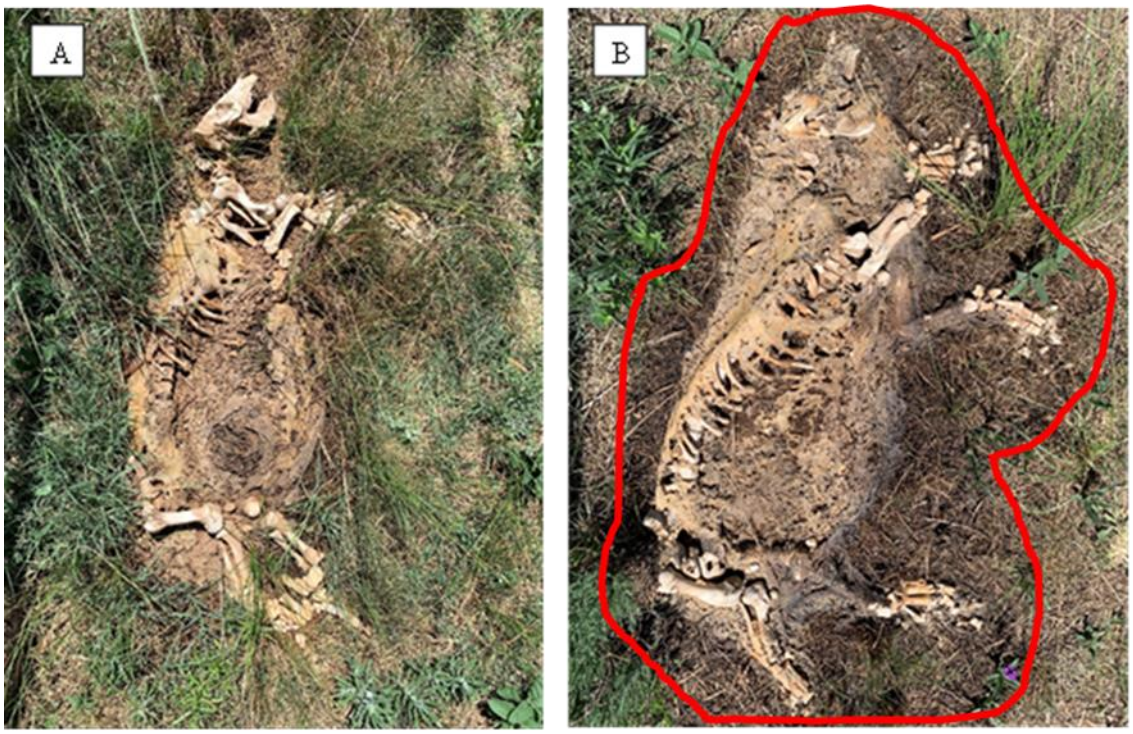

Figure 4.11: **A)** Control pig 3 on PMI 82 days (1697ADD), and **B)** experimental pig 5 on PMI 82 days (1697ADD), post-rehydration of Trial 3 displaying a greater CDI (dark stain on ground surrounding the carcass outlined in red).

Indirect rehydration

Although efforts were put towards deploying pigs in the dry seasons in the Highveld, natural rehydration or indirect rehydration occurred during the experimental phases of the research project. Indirect rehydration was seen to effect both the decomposition rates of the experimental and control carcasses of all four trials. The tarps prevented direct rehydration from natural rehydration, however, changes were still observed due to indirect rehydration likely from the soil e.g., increased insect activity and re-initiation of decomposition, post-rehydration, as indicated by changes in TBS. The different trials had rainfall occurring during different phases of decomposition. The trials all experienced rainfall throughout all the stages of decomposition, although most were very small showers. The indirect natural rehydration was associated with an increase in the rate of decomposition at ±600ADD for all the pigs of Trial 2 (Figure 4.4A & B). Trial 3 demonstrated an increase in the rate of decomposition observed for EP5 (ADD 500-600). In Trial 4, the rainfall which occurred on PMI day 32 (673ADD) contributed to the wetting event of the two experimental pigs (Figure 4.8A & B), which led to a re-initiation in the rate of decomposition. Even though the carcasses were not controlled in their rehydration, the rehydration took place during the stagnant phase of decomposition. It is also important to note that during rainfall, the average daily temperature dropped (Figure 4.3B, 4.4B, 4.5B & 4.8B).

4.3 Insect activity

As previously stated, decomposition is mainly driven by temperature and insect activity. The final objective of this research was to assess the subsequent effect that rehydration has on insects and if it would lead to a re-colonisation of the carcass. The assessment of insect activity was quantified by applying an accumulative regional score of presence or absence of insects on the carcass. Each trial has a graphical representation of the insect activity over the corresponding ADD (Figures 4.3C, 4.4C, 4.5C, & 4.8C).

All the pigs followed a similar pattern of insect colonisation. The fly larvae (*Diptera*) were the first invertebrate colonisers (*Calliphoridae* [blowflies], *Sarcophagidae* [flesh flies], and *Muscidae* [house flies]) in the mouth and anus during the early stage of decomposition. The maggots were present under the skin of the carcasses and mainly digested the muscle and fat tissues, avoiding the skin during the early stage of decomposition. When comparing the initial insect activity between the four trials, Trial 2 experienced a notably decreased insect activity

during the fresh to early stage of decomposition (100 to 700ADD) (Figure 4.4C). Trial 1 had a delayed onset of insect activity ±100ADD (Figure 4.3C), Trial 3 also had a delayed onset with all pigs having colonisation of only 1 area (Head) at ±200-300ADD (Figure 4.5C), and Trial 4 experienced immediate insect activity (Figure 4.8C).

Desiccated skin was present on all the pig carcasses when they stalled in advanced decomposition. Hide beetles were observed (*Coleoptera Dermestidae*) to remove the remaining skin (Figure 4.12B). Other invertebrate colonisers of the *Coleoptera* species were observed, including chequered beetles (*Cleridae*), skin beetles (*Trogidae*), and cheese skippers (*Piophilidae*).

The general trend observed over the entire experimental trials (Figure 4.3, 4.4, 4.5, & 4.8), included a decrease in insect activity when the temperature decreased. Insect activity was seen to decrease after a lot of rainfall as well as directly after the direct rehydration, however the insect activity then re-instated for a short period of time. The insects observed post-rehydration mainly included Hide beetles. Flies were present post-rehydration and after indirect rehydration events but there was no maggot re-colonisation and migration observed in or around the carcass. The increase in activity was also observed after smaller bouts of indirect natural rehydration and increased average daily temperatures. It was also observed that, since the carcasses were only covered at the top, hydration from natural rehydration resulted in the soil surrounding the carcass to become moist with a subsequent increase of beetles on the carcass (Figure 4.12).

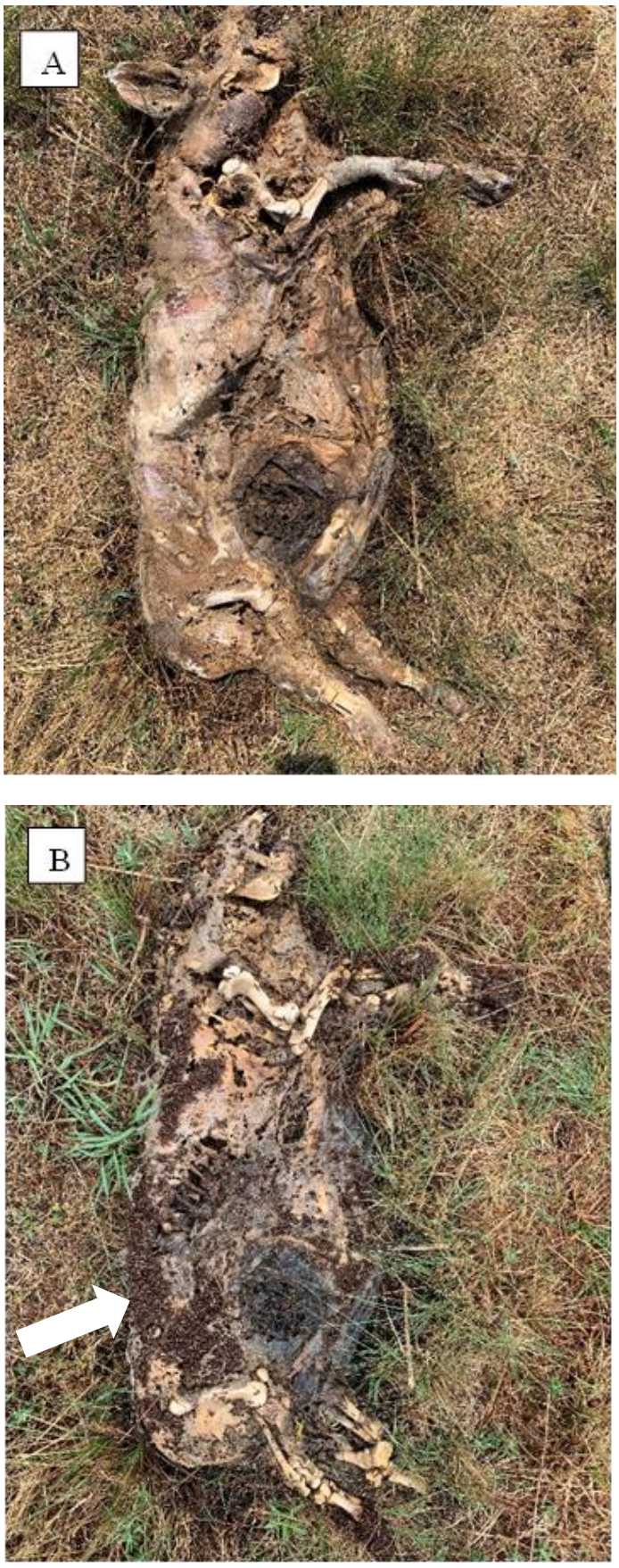

Figure 4.12: **A**) Control pig 3 (CP3) on PMI day 47 (925ADD) and **B**) CP3 on PMI day 50 (989ADD) colonised by beetles (*Coleoptera dermestidae*) indicated by the white arrow, post indirect natural rehydration resulting in soil hydration.

Chapter 5 Discussion

This study explored the effects of rehydration on decomposition and its subsequent effects on insect activity. An improved understanding of the different effects of environmental factors on the rate of decomposition results in greater accuracy of PMI estimation methods. South Africa faces the unfortunate reality of a high number of unidentified human decedents who remain unidentified (Keyes et al., 2016; Brits et al., 2020). When remains are found beyond the point of identification, the study of taphonomy is applied, which assesses the environmental factors that play a role in how far a body has decomposed and links this to a timeline post-death. Once a timeline has been established, this information can narrow down individuals that have been reported missing and narrow down a possible identity (Megyesi et al., 2005).

Forensic taphonomy is a relatively young discipline, with many of the PMI estimation methods looking at a single environmental factor, such as temperature (Megyesi et al., 2005). Temperature is a constant variable that plays a role in the rate of decomposition. However, the ADD/TBS PMI estimation method, created by Megyesi et al. (2005) and modified by Moffatt et al. (2016), has not displayed high degrees of accuracy across different seasons and differing environments (Myburgh et al., 2013; Marhoff-Beard et al., 2018; Forbes et al., 2019). This suggests that other forces, such as rainfall as an example, act on the decomposition rate. Weather conditions are impossible to control and difficult for researchers to isolate in order to understand their specific role in the decomposition process however, a clearer understanding of these factors and their influence on the rate of decomposition is necessary. Therefore, this research study set out to artificially rehydrate pig carcasses to assess the effect of rehydration (rainfall) on decomposition. This research indicated a change in the rate and progression of decomposition after a carcass has been rehydrated and changes in insect activity based on the moisture present during the decomposition cycle. The findings of this study will be discussed in three sections: decomposition (rate of decomposition and the effects of rehydration on the rate of decomposition) and insect activity.

5.1 Decomposition

Decomposition was observed over the trial phases, with the progression of decay recorded in terms of TBS. This data was then correlated to each pig's corresponding ADD for ease of

analysis (Megyesi et al., 2005; Keough et al., 2017). The following section will discuss decomposition pre-rehydration, post-rehydration, indirect-rehydration, and temperature.

5.1.1 Pre-rehydration decomposition

Decomposition is highly affected by different environmental factors during the decay process. The TBS/ADD graphs represent a sigmoidal distribution for all the trials in this current research, with an initial rapid increase in the TBS over the subsequent ADD followed by a plateau period where the TBS remains the same for a period and then proceeds with TBS increasing until the carcasses decomposition plateaus once more. Myburgh et al. (2013) conducted the baseline data for decomposition in the Central Highveld area, Gauteng, South Africa. The progression of decay displayed a sigmoidal curve, with an initial rapid rate followed by a plateau and then a slowed rate of decomposition (Myburgh et al., 2013). These findings display that decomposition progresses in a relatively uniform manner regardless of the progression and rate of decay affected by the environment.

The restriction of cage availability resulted in two distinctive groups of trial deployments. Trial 1 was separated into the cooler deployment, and the other trials (Trial 2, 3, and 4) were grouped in the warmer deployment, having experienced an average temperature of ±5°C warmer than the cooler deployment (Table 4.2). This research found that all the carcasses deployed in the first trial during cooler temperatures experienced a faster initial rate and then stalled in their decomposition an approx. month after deployment (Figure 4.3A). Whereas the other carcasses in Trials 2, 3, and 4 only stalled after two to three months post-deployment (Table 4.3). The Trial 1 pig carcasses (EP1, EP2, and CP1) displayed the greatest rate of decay through the initial stages of decomposition. The Myburgh et al. (2013) study observed the linear progression of initial decay. Typically, colder conditions resulted in a slower rate of decay (Arnaldos et al., 2004; Finaughty, 2019; Giles et al., 2020), however, similar rates of initial decay have been observed between winter and summer carcasses in the Highveld (Myburgh et al., 2013). Furthermore, it was later established that the pigs from Trial 1 were kept in a cold room overnight to allow simultaneous deployment. This resulted in the carcasses being exposed to a lower temperature prior to deployment. All the pigs within Trial 1 experienced similar conditions, therefore, comparison of the experimental to the control pigs was possible. Trials 2, 3, and 4 pig carcasses were deployed on the day of death. The possible reason behind the Trial 1 experiencing a far greater initial rate of decomposition may be due to noticeably more rain occurring during the initial stage of decomposition. The carcass may have been exposed

to more humidity due to the rainfall and promoted bacterial and insect activity (Jaworski & Hilszczański, 2014). Alternatively, due to the remains having been exposed to cooler temperatures prior to deployment the sudden introduction of warmer environmental process may have kick started the bacterial breakdown of the remains, as bacteria proliferates given the right conditions (Pascual et al., 2017; Iancu et al., 2018).

When comparing the rate of decomposition between the cooler and warmer trials, the PMI and ADD only differed slightly in reaching a TBS of 17. Like in Myburgh et al. (2013), a similar rate and decay progression (linear) was observed for both seasonal trials until TBS 17. The progression of decomposition for all the trials being clustered during the early stages, unlike the latter stages (Figure 4.9). After deploying 30 pigs over 232 days, Myburgh et al. (2013) noted that all pigs in the sample initially experienced a similar decomposition rate and then stalled in their decomposition almost wholly. There are possible reasons for the differences observed between the initial rates from the fresh to the early phase of decomposition other than temperature playing a role. The warmer trials (Trial 2, 3, and 4) displayed greater PMI and ADD values to reach a similar TBS of 17. When drawing comparisons between the warmer trials, the decomposition rate was slower for the Trial 2 and 3 samples. In contrast, Trial 4, having not been deployed too long after Trial 2 and 3, experienced a faster initial rate of decay to reach a TBS of 17. The rate of Trial 4 decay experienced a higher environmental temperature during the fresh to the early phase of decomposition, similar to that of Trial 1 (Figure 4.3B & 4.8B) (Arnaldos et al., 2004; Finaughty & Morris, 2019; Giles et al., 2020). The similarities between Trial 1 and Trial 4 consist of a linear and rapid progression through the initial stage of decomposition. The two trials differed into the advanced stage of decomposition where Trial 1 endured decreasing temperatures as the study transitioned from Autumn into Winter which may have resulted in the clustered distribution of TBS values toward the end of the decay cycle. Trial 4 however experienced consistent warm temperatures through all the stages of decay unlike Trial 2 and 3, these trials transitioned out of winter into spring and subsequently experienced the colder temperatures in the early stage of decay when insect activity and bacterial proliferation is maximum (Goff, 1993). A general trend where warmer seasons' carrion decomposes faster over all (Arnaldos et al., 2004; Finaughty & Morris, 2019; Giles et al., 2020) indicates the possible effect of temperature and rainfall working together in speeding up the initial rate of decomposition. Differences in humidity levels in the form of indirect rehydration, insect activity, possible bacterial infections or treatment prior to death, and body size may affect the decomposition rate (Anderson &

VanLaerhoven, 1996; Tabor et al., 2004; Bachmann & Simmons, 2010; Voss et al., 2011; Sutherland et al., 2013; Jaworski & Hilszczański, 2014; Matuszewski et al., 2014; Roberts et al., 2017; Wang et al., 2017).

Another trend was observed amongst the sample group, where the larger carcasses displayed a different rate of decomposition (Figure 4.4A & 4.5A). Between the samples, it was noted that the pigs of a similar weight decomposed at a similar rate. The larger carcasses in the trials decomposed at a slower rate and the smaller pigs. The larger body size results in more tissue for both bacterial and insect activity to break down all the biomass (Kneidal, 1984) and hence, a slowed rate of decomposition. Body size is noted to alter the rate of decomposition of carrion (Sutherland et al., 2013). The current research project observed notable differences in the rate of decomposition between two larger pigs within the sample group. The larger pigs weighed ±90kg (EP3) and ±100kg (EP6) with EP6 reaching a TBS of 17 in nearly a third of the time and half the ADD compared to the initial rate of EP3. The EP3 was exposed to similar environmental conditions as EP6, deployed 28 days prior. EP3 did not skeletonise fully and remained in the advanced stage of decomposition unchanged for most of the experimental phase, with minimal bone exposure and visible insect activity.

The slow rate of decay observed in EP3 may be due to the initial temperatures hindering bacterial or insect activity, as note by Mann et al. (1990). Trial 2 experienced a minimum temperature of 2°C. In contrast, Trial 3 experienced a minimum temperature of 4°C, a delayed onset of insect activity, environmental temperatures may have altered the colonisation of invertebrate scavengers on EP3. As noted for EP3, EP4, and CP2, lacked extensive maggot colonisation and migration. Along with colder temperatures, windy conditions may have led to the dehydration of the pig carcasses like that of Finaughty & Morris, (2019). This desiccation would lead to the depletion of nutrients for bacteria to feed on (Campobasso et al., 2001).

Although natural rehydration did occur during the experiment, the carcasses were protected by the tarps, and all the carcasses reached a point of desiccation during the experimental phase. As predicted, the carcasses stalled in their decomposition (TBS values 19-25) and the experimental carcasses were subsequently rehydrated.

5.1.2 Post-rehydration

The second objective aimed to address the effects of rehydration on the rate of carrion decomposition. Rehydration took place during the advanced phase of decomposition. This stage of decomposition is characterised by the remains entering a state of desiccation. Desiccation is the drying out of carrion tissue and inhibition of bacterial and insect activity resulting in cessation of active decomposition (Galloway et al., 1989; Campobasso et al., 2001). The following section will discuss the effects noted post-rehydration on the rate of decomposition and subsequent effects observed after rehydration.

After rehydration, the experimental pig's decomposition rate for all trials increased (±2.62 TBS over ±10 days) compared to the control samples. The severity of differences seen between the initial rate of decay between the warmer and cooler trials as Archer (2004) concluded is that higher temperatures and more rainfall result in a faster rate of decomposition. The effects of rehydration changed the typical progression of decomposition, i.e., the experimental pigs had a re-initiation of decay after direct rehydration and then entered the stagnant phase again. The control pigs of the experiment enter the stagnant period remaining this way until the end of the study. Slight increases in TBS scores were also observed after cases of indirect rehydration for both the experimental and control carcasses, as discussed below. It has been proposed that after rehydration, the tissue becomes palatable once more for the insects (Finaughty & Morris, 2019), and the insects re-colonise the remains (Archer, 2004; Myburgh et al., 2013).

Trial 4 experienced natural, direct rainfall due to the removal of the tarp by excessive winds, during which time the experimental carcasses (EP7 and EP8) were rehydrated to the point where the flesh changed colour (Figure 4.6 & 4.7). This is seen in other research where rehydration (exposure of humidity) of desiccated tissue appears to change the visual appearance of the carcasses (Suckling et al., 2016). The colour changes of tissue appeared devoid of pigmentation, white or ashen, as has been observed in arid environments previously browned, blackened tissue (Byard et al., 2020). The rate of decomposition of the naturally rehydrated carcasses displayed accelerated decay for EP7, but this was not as prominent for EP8. Another possible comparison could be drawn between dark and leathery tissue due to post-mortem tissue drying that has been saturated with water, causing a colour change to a lighter appearance. Victims whose remains are submerged in water display a change in the colour of their skin (Dalal et al., 2020).The study noted that the pig carcases that had been submerged had experienced sloughing of the skin and turning “greyish” in colour. Therefore,

it is noted that the possible time of exposure to rainfall affects the rehydration of the dried decomposed skin to change colour attributed to possible saturation of water. If remains are found to have this whitened saturate colour change, depending on the location (next to a body of water) the remains are found in, it may affect the interpretation of post-mortem events.

Another qualitative observation made during the study revealed that carcasses that had been directly rehydrated displayed a greater CDI than those not rehydrated (Figure 4.6 and 4.7). The presence of CDIs is not uncommon in taphonomic research (Fancher et al., 2017; Barton et al., 2020; Cogswell & Cross, 2021). Cogswell and Cross (2021) noted that CDIs differed based on the surfaces that the carcasses were placed on. The research looked at the effect of differing surfaces e.g., concrete, grass, and gravel (Cogswell & Cross, 2021). Regarding rainfall, Cogswell and Cross observed CDIs present around a carcass on a grass surface to persist after being exposed to rainfall compared to other surfaces, such as gravel and concrete. Greater CDIs are generally expected with larger carrion due to more decomposition fluids (Kneidal, 1984). However, the size of CDIs and its relation to the biomass of a carcass has not been directly assessed in the literature, only the intensity of the nutrient deposits and lateral spatial extent of CDIs in cases of scavenger activity compared to remains that had not been scavenged on (Aitkenhead-Peterson et al., 2012; Fancher et al., 2017; Barton et al., 2020). Evaluation of CDI through soil chemistry is currently being explored as a method of PMI estimation (Fancher et al., 2017; Barton et al., 2020). The method explores the presence of different compounds in the soils and CDI surrounding the body or possible decomposition site and is noting the absence and present of differing soil chemistry over time to estimate PMI (Fancher et al., 2017). The findings of this research suggest the need for possible research specifically related to the extent of the effect of rehydration on CDI. Research should also include how the size and depth of the cadaveric nutrients into the soil could affect the interpretation of PMI estimation after rehydration.

When remains desiccate due to a lack of moisture, the remains enter a state of stasis. Thus, the rate of soft-tissue decay is strongly reliant on the environment (Dirkmaat et al., 2008). The most used method of PMI estimation attributes temperature for 80% of the variation seen in physical changes occurring during the decomposition process (Megyesi et al., 2005; Marella et al., 2013; Finaughty & Morris, 2019; Kadej et al., 2020; Lennartz et al., 2020). However, in the case of rainfall its effects are not fully understood. The standardised methods of PMI look solely at temperature (Megyesi et al., 2005; Schiel, 2008; Moffatt et al., 2016; Jeong et al.,

2020), and the noted effect seen by rehydration needs to be considered when observing remains and the environment they have been exposed to over the PMI. This supports the findings of Myburgh et al. (2013), where, once a body has entered this "stagnant period", rainfall would mask this period, and estimation of PMI would be incorrectly interpreted to be shorter.

5.1.3 Indirect rehydration

Indirect rehydration occurred during the experimental phase, where the soil around the cages was rehydrated by natural rehydration. This rainfall occurred early at the start of the decomposition cycle of the first Trial and towards the end of the decomposition cycle for Trials 2, 3, and 4. The stages the carcasses were at in their decomposition cycle, during the indirect hydration events, displayed different effects on the progression of decomposition. In some cases, the extent of the indirect natural rehydration had no direct effect when the carcass (EP3) was stalled in decay as no change was observed until after the direct rehydration. Other carcasses experienced an increased rate of decay and insect activity, such as EP1, EP2 and the control pig (CP1) during the fresh to the early stage of decomposition. The trend of increased decomposition rates at the beginning of the decay cycle is expected (Myburgh et al., 2013; Finaughty & Morris, 2019). This may also explain the initial increased rate of decay for Trial 4 pigs experiencing indirect natural rehydration during the early phase of decomposition whilst experiencing warmer temperatures. This may indicate that both indirect and direct rehydration influence the rate of decomposition due to the ground where the carcass lay becoming hydrated or exposed to increased humidity (Galloway et al., 1989; Mann et al., 1990; Giles et al., 2020).

Humidity, although out of the scope of analysis for this research project may have contributed to the rate of decomposition increasing when the carcasses where exposed to indirect natural rehydration. A positive and negative effect of humidity on insect is noted (Jaworski & Hilszczański, 2014). Higher temperatures and low humidity resulted in a shorter developmental period for larvae observed in Jaworski and Hilszczański (2014). However, increased humidity can also slow decomposition into the skeletonisation stage, which occurs faster in dry and hotter conditions (Cardoso et al., 2010; Marella et al., 2013; Finaughty & Morris, 2019). The tarps covering the cages may also contribute to the humidity of the area surrounding the carcass and will require further investigation to understand the possible influence it has on the rate of decomposition.

5.1.4 Temperature

The greater environmental temperatures recorded during this research resulted in increased rates of decay in both the cooler and warmer trials as critical thermal energy is required for the bacteria and insect activity to take place (Mann et al., 1990; Vass et al., 1992; Shean et al., 1993; Megyesi et al., 2005; Finaughty & Morris, 2019). During rainfall events, the average temperature dropped for all the trials. This finding is consistent with field research observing the different effects of biotic factors on the rate of decomposition (du Toit, 2019; Finaughty, 2019; Finaughty & Morris, 2019; Spies et al., 2020). During rainfall, as seen in the literature (Megyesi et al., 2005), insect activity is directly affected by the associated lower temperatures. During direct artificial rehydration, the temperature change is not observed and, therefore, does not accurately represent a realistic scenario. However, it does allow a unique opportunity to study the isolated effects of rehydration on decomposition.

5.2 Insect activity

The third objective of this research set out to assess the insect activity after a pig carcass has been rehydrated. The insect activity observed during the different stages of decomposition will be discussed, observing changes that have occurred before and after rehydration and the subsequent effect on the rate of decomposition. The insect activity was noted during the site visits and represents a broad over view of the insect activity experienced by the carcasses. In order to fully assess the insect activity future studies should implement remote 24-hour monitoring.

The progression, colonisation, and onset of insect activity was noted throughout the study for all pigs. The pattern of insect colonisation was noted to be similar for all the carcasses regardless of the timing and climatic conditions (warmer and cooler trials). Insect activity followed the typical pattern of insect colonisation with fly activity and maggot colonisation of the head and anus, progressing with larvae/maggot migration over the trunk region. The progression of the insect colonisation was like that typically observed in decomposition studies (Keough et al., 2017; Finaughty, 2019; Spies et al., 2020). The progression was consistent amongst the pig carcasses, excluding EP3, having only observed limited maggot colonisation and migration possibly contributing to the slowed rate of carrion. The pre-rehydration insect colonisation and pattern of progression was largely consistent between the carcasses allowing decomposition to progress with unhindered insect access.

The onset of insect activity was noted to be present within one day PMI for most of the pigs. The studies occurring during warmer initial periods of the decay cycle displayed a faster onset of insect colonisation (Arnaldos et al., 2004; Meyer, Anderson & Carter, 2013; Giles et al., 2020). However, in Trial 2 a delayed onset of first wave of invertebrate colonisers was observed, notably for EP4 and CP2 (Figure 4.4C). Trial 2 experienced an average temperature of ±10°C for the first seven days PMI (±75ADD). It was observed that insect activity decreases with the lowered temperature, consistent with the proposed base temperature for insect activity of 10°C (Catts, 1992). The decreased temperatures during the fresh stage of the decomposition experienced by Trial 2, is a proposed reason for the delayed onset of insect activity and resulting in a slower rate of decomposition.

The fly/maggot activity ceased once the early stage of decomposition ended and the beetle activity increased once the carcasses entered the advanced stage of decomposition. After the tissue dried out, flies no longer deposited larvae on the carcass. Similarly seen in the literature due to the flesh no longer containing enough moisture to promote maggot growth and be palatable for maggot consumption (Ayers, 2010; Finaughty & Morris, 2019). The hide beetles are biologically equipped to consume a harder material such as dry skin/hide (Schroeder et al., 2002). However, the Hide beetle larvae are very susceptible to environmental factors and cessation of *Dermestidae* can occur once the tissue is entirely devoid of moisture or subject to waterlogging and predation (Archer & Elgar, 1998). The current research suggests a possible explanation for increased visibility of beetles' post-rehydration may be due to an excess of water creating puddles on the carcass forcing the beetles inside the carcass to rise to the surface and become visible to the researcher.

Insect activity stalled for the control carcasses in the advanced to the skeletonisation stage of decomposition. Insect activity appeared to decrease when the average daily temperature decreased. A drop in average daily temperature was also observed when rain fell. When looking at the effect the insect activity had on the decomposition of the sample, minimal insect activity was observed during the colder days (Figure 4.3C & 4.4C), and the pigs remained fresher for longer. This is expected since colder temperatures are associated with less insect activity (Megyesi et al., 2005). Alternative patterns of decay may be observed based on post-rehydration insect activity during the colder temperatures, and possible different rates of decay observed when the carcass is exposed to colder temperatures and rehydration.

Insect activity was altered in the experimental carcasses post-rehydration. Flies were present after rehydration but no visible maggot masses. A similar finding of rehydrated tissue was observed however no return of fly activity was visible, although, other scavengers returned to the carcass (Ayers, 2010). This research observed that after post-rehydration, more *Coleoptera* species presented on the remains, such as hide beetles (*Dermestidae*). Hide beetles are associated with desiccated tissue leaving holes in the tissue where the insects have eaten through the desiccated hide helping the remains to skeletonise (Marella et al., 2013; Finaughty & Morris, 2019; Kadej et al., 2020). Schroeder (2002) observed that optimal conditions for *Dermestidae* are that of a warm and dry environment. The hide beetle was the main coloniser pre- and post-rehydration. *Dermestidae maculatus* are noted to become the dominant invertebrate scavenger present middle to late decomposition, larvae are present once the carcass has dried out (Richardson & Goff, 2001). The dry tissue of the advanced stage of decomposition supplied palatable tissue for the beetles to feed. The wet conditions induced by the rehydration may have initially deterred the hide beetles. Explaining a decrease in initial colonisation after rehydration events as observed in EP4.

The experimental pigs in Trial 4 were rehydrated by natural rehydration, and an increase in insect activity was noted for EP7 and a slight but immediate increase in insect activity for EP8. Flies were present, but no oviposition was observed. Flies are associated with the fresh to the early stage of decomposition where the tissue is still moist (Clark et al., 1997). A possible reason for the re-attraction but no larvae production may be the tissue becoming rehydrated and releasing VOCs. A study conducted by Forbes et al. (2014), displayed the pig carcasses to have an initial rapid increase in VOCs during active decay and a second increase in during the later stages of decomposition. A large amount of rain fell during the study preceded by a second peak in VOCs (Forbes et al., 2017). The author noted the tissue of the carcass to be moistened after a period of desiccation and evidence of pupal cases and recently emerged flies and a simultaneous increase in VOCs. The VOCs of this current research were not recorded, but may provide possible reasoning for the abundance of insect activity post-rehydration. The fly population subsequently increased post-rehydration but as mentioned no maggot re-colonisation was observed. The conditions of the carcass may still not be palatable enough for maggots to thrive (Finaughty & Morris, 2019). The findings suggest that the amount of rain is possibly a factor in the degree of recolonisation or cessation of insect activity.

Although the carcasses were covered from the top from direct rainfall, the carcasses were exposed to moisture absorbed by the soil. It is possible that moisture was absorbed by the carcasses in both the control and experimental groups from the soil since recolonisation of beetles and flies was observed after natural rehydration (Figure 4.12). A possible explanation for the sudden increase in visible Hide beetle larvae after the artificial rehydration may be due to the waterlogging, as mentioned by Archer (1998), where by the larvae move to the top of the carcass to escape fatality. A closer observation of the location of the hide beetles prior to rehydration may inform the observations present in this current research project. The beetles may not be re-colonising the carcass but may not be visible to the researcher prior to rehydration.

The moisture of the soil has a significant effect on the rate of decomposition through the interaction with microbial activity (Carter et al., 2010). The moisture is also directly correlated to insect activity, which Finaughty & Morris (2019) observed. In a natural setting, the timing of rainfall would affect the oviposition of the fly larvae should it occur during the early phase of decomposition (Castner, 2010; Simmons et al., 2010; Al-Qahtni et al., 2020). If the rainfall occurred during the latter part of early decomposition after maggot colonisation, the maggots would shelter themselves against the effects of the rain. The effects of rainfall would either lead to insects continued internal feeding on the carcass or have the insects and larvae be washed away by severe weather (Mann et al., 1990; Kelly et al., 2009). Therefore, in the case of indirect rehydration, insect activity varies based on the amount and length of time that rehydration/rainfall takes place. Future research into both the duration and amount of rainfall needs to be explored. Insects are a main driver of decomposition, however the severity of their effect on the rate of decomposition is highly influenced by the environmental conditions the remains are found (Mann et al., 1990). Therefore, great consideration of environmental conditions should be taken as their effects may alter the interpretation of PMI estimation when entomological methods are being utilised.

5.3 Limitations and Future Recommendations

Limitations of this research project were introduced due to unforeseen/unavoidable circumstances. The study took place over a Global pandemic caused by the spread of COVID-19, which delayed the initiation of this research project. The initial timeline would have allowed two sets of deployments over two winter seasons of 2020 and 2021. However, due to

the subsequent delays, the carcasses were only deployed during the winter of 2021, limiting the number of trials deployed and limiting the use of statistical analysis.

Due to time, budget, and availability constraints, the sample size was limited. However, with a deployment of 12 pig carcasses, eight experimental and four control, similar trends were observed amongst most of the sample group. The sample size was not large enough to conduct statistical analysis but is a comparable sample size within taphonomic research (Sutherland et al., 2013; O'Brien et al., 2017; Dibner et al., 2019; Keyes et al., 2019; Spies et al., 2020). Due to the intra-specific variability, a definite trend will be tough to observe in decomposition sciences. There are too many factors at play to continuously control and replicate an experiment. A suggested solution to the sample size would be a longitudinal study over multiple years and seasons.

The conditions the study sample were exposed to before deployment have suggested possible effect on the decomposition rate. For example, the pigs used for this research project died from similar diseases such as *Lawsonia intracellularis* and *Haemophilus parasuis*. Disease has been described to affect the rate of decomposition (Campobasso et al., 2001; Hayman & Oxenham, 2016), yet this has not been tested on pig models that have had possible antibiotic treatment prior to death. Antibiotics are known to kill the bacteria in the gut biome (Panda et al., 2014; Mikkelsen et al., 2015), and since bacteria are a major driver for the fresh to the early stage of decomposition, the rate may have been affected (Pascual et al., 2017; Iancu et al., 2018). The effects of antibiotics on living humans' gut microbiome have been explored extensively (Looft & Allen, 2012; Francino, 2016; Zhang et al., 2019; Yang et al., 2021). However, there is possible room for an investigation into the post-mortem role of dysbiosis (reduced diversity in the gut microbiome) in the rate of decomposition. A suggested study may address the rate of decomposition between a healthy sample of pig carcasses and a sample dying of natural causes, such as the diseases mentioned above. Insects' cross contamination present due to a staggered multi-carcass deployment was attempted to be controlled for by spacing the carcasses at least 10 meters apart in any direction. The cross contamination of insects is difficult to control and has to be considered when analysing the insect data.

Cages had to be constructed to limit scavenger activity and access of direct rainfall. Limiting natural scavenging does alter the forensic realism of the findings (Miles et al., 2020). However, this research project set out to assess the baseline effects of rehydration on decomposition and the elimination of scavenger activity allowed for a focused analysis of the subsequent effects

of rainfall. Due to lack of availability and time constraints, secondary cages were constructed that differed from the primary cages, perhaps introducing an altered environment for decay to take place. For example, the height of the secondary cages differed from the primary cages. The secondary cages had stakes to change the angle of the tarp to allow for rain to run off (Figure 4.2), unlike the flat roof of the primary cages (Figure 3.2). However, the secondary cages aimed to meet the same criteria as the primary cages by allowing for unhindered insect activity and sufficient airflow for the carcass. Humidity may have been a factor at play introduced by the transparent tarp; however, all the carcasses experienced the same conditions and as the tarp did not hinder airflow this was not considered to be a defining variable. Although should be explored further to understand the effects of humidity, as well as rehydration. The secondary cages also covered the carcass from direct rainfall. However, it did allow for the Trial 4 experimental carcasses to become rehydrated due to unforeseen winds removing the plastic tarp from where it had been fastened over the cage. Fortunately, the timing of the rehydration aligned with the research criteria and thus was not considered detrimental to the research project. However, it is acknowledged that the "naturally" rehydrated carcasses may have been exposed to more water than the artificially rehydrated carcasses for an extended duration. The differences between the natural and artificial rehydrated carcasses highlights that the amount and duration of water/rainfall can alter the progression and rate of decay and warrants further research.

Initially, only three cages were constructed, limiting the number of pigs deployed at one time. The delay in cage production resulted in deployment later in the season than anticipated, and continued decay exposed the pigs to more natural rehydration than expected. This limitation caused from the need for the cages to restrict scavenger activity and cover the carcasses to prevent direct rehydration in natural rehydration. However, the carcasses were rehydrated by indirect rain and absorption from the soil surrounding the carcass. A further adaptation to the experimental design may include a mesh placed under the carcass to allow sufficient insect and bacterial access for surface decomposition while limiting the absorbed ground moisture. In order to fully control for artificial rehydration, a possible methodology could be set in place to prevent other forms of external hydration. Alternatively, to include a larger covering over the carcass to prevent the ground moisture from reaching the carcass. The rehydration method may have limited the results by the implementation of artificial rehydration only taking place once during the decay cycle. This is unlike indirect rehydration events occurring multiple times during all the trials. However, the indirect rehydration also showed to rehydrate the carcasses

and provided the researcher with valuable information regarding alternative effects of rehydration.

The carcasses were rehydrated after having dried out and entered the advanced stage of decomposition. The study was limited to the analysis of rehydration during the advanced decomposition stage, a stage selected based on findings of previous research in the region conducted by Myburgh et al. (2013). However, the analysis and further scientific enquiry into rehydration at difference stages of decomposition may be informative to the further effects of rehydration on the rate of decomposition and possible effects on the estimation of PMI.

The rehydration of the carcass with different amounts and durations at different stages of decomposition might have different effects of the pattern of insect colonisation and rate of decomposition. Little is known about this, and research into the effect of rehydration during different phases of decomposition is warranted. More in-depth knowledge of rainfall in the region may better the current method of 10L (equivalent of 10mm of rainfall) poured over the carcass in a relatively short period. The 10mm of rain may fall all at once, introducing a different effect on tissue rehydration and insect activity than 10mm of rainfall over an extended period as observed in the natural wetting of Trial 4's experimental carcasses.

In summary, this experimental study revealed many avenues of research for future exploration. All these suggestions will help better the accuracy of PMI estimation through experimental design and identification and hopefully create a shift in focus of the aspects that affect the rate of decomposition.

Chapter 6 Conclusion

This research was set up to assess the baseline effects of rehydration on decomposition and analyse the subsequent effects on insect activity. The study took place in the Central Highveld, Gauteng, South Africa. The purpose for this research was to investigate the possible need for increased consideration of rehydration as a factor influencing the rate of decomposition ultimately resulting in more inclusive and accurate estimation of time of death in a forensic investigation through taphonomic enquiry. This study's aim was achieved by deploying 12 pig carcasses, with eight experimental pigs rehydrated after reaching a state of stasis in an advanced stage of decomposition. The taphonomic data included TBS, invertebrate scavenger activity, and prevailing weather data. Notable differences were observed in the insect activity, progression of decomposition and its associated rate after direct rehydration, compared to the control carcasses.

- Rehydration increased the TBS values after the carcasses entered an initial state of stasis and created a greater CDI around the experimental carcasses that had been rehydrated compared to the control sample.
- Insect activity was altered not only by artificial rehydration but prevailing weather conditions.
- The carcasses stalled in advanced decomposition due to lack of moisture resulting in desiccation. When rehydration occurred, insect activity was revived and continued for the entire trial phase of the experimental carcasses while it stalled in the control samples.
- When direct rehydration occurred naturally, changes in skin colour was noted and a distinctive increase in hide beetle colonisation.
- When the soil below the carcasses was indirectly rehydrated, a recolonisation of hide beetles was also observed for all carcasses in advanced decomposition.

In conclusion, this study suggests that rehydration influences insect activity, progression of decomposition and its associated rate. The findings of this research have provided baseline data to address a gap in the current literature relating to the effects of rehydration on decomposition. Therefore, must be taken into consideration when a body is found. Rehydration could mask the PMI of the remains when calculated based on the degree of decomposition, resulting in an estimation of a shorter PMI. The accuracy of PMI estimation is essential in identifying unknown individuals (Megyesi et al., 2005) and the positive identification of unknown

decedents will move society one step closer to restoring social justice and provide much-needed closure to the family of the otherwise "pauper" individual.

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Appendices

Appendix A – University of the Witwatersrand Research Ethics Approval

ANIMALS RESEARCH ETHICS COMMITTEE (AREC)

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

STRICTLY CONFIDENTIAL

CLEARANCE CERTIFICATE NUMBER: 2020/05/05/O

APPLICANT: Ms C du Toit
School: School of Anatomical Sciences; Department: N/A; Location: GHB Farm, Forensic Anthropology Body Farm (FABF), University of Pretoria

PROJECT TITLE: The Influence of Rehydration on porcine carcasses (*Sus scrofa*), assessing Decomposition in the Highveld Region of South Africa.;
Category: O; Species and Numbers involved: *20X 50-100kg male and female domesticated pigs (sus scrofa)*

Approval is hereby given for the use of animals for the research project named above and described in the application reviewed by a quorate meeting of the AREC held on 26 May 2020. This approval remains valid until 23 Jun 2022 and is conditional to the following (if blank there are no special conditions):

Condition 1	Condition 2	Condition 3	Condition 4

All material changes to the approved research must be reported to the AREC before they are implemented. Failure to do so will invalidate this clearance certificate.

An annual progress report must be provided to the AREC.

The use of these animals is subject to AREC guidelines on the use and care of laboratory animals, is limited to the procedures described in the application and is subject to additional conditions listed below:

I, the Chair of the AREC (or my designated representative) am satisfied that the proposed research is ethical as judged by local law, international standards and University policy.

Signed: _____ _____ Date: ___25/06/2020_____

(Chairperson of the AREC)

I am satisfied that the persons listed in this application are competent to perform the procedures described in the application, in the context of Section 23 (1) (c) of the veterinary and Para-veterinary Professions Act (19 of 1982).

CC: Student supervisor: «Title1» «Initials1» «Supervisor_surname»
Director Central Animals Service: Dr Kim Jardine

ANIMALS RESEARCH ETHICS COMMITTEE (AREC)

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Signed: _ _____ Date: _26/06/2020_____

(Registered Veterinarian)

CC: Student supervisor: «Title1» «Initials1» «Supervisor_surname»
Director Central Animals Service: Dr Kim Jardine

Appendix B – University of Pretoria Research Ethics Approval

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science
Animal Ethics Committee

7 December 2020

Approval Certificate with Conditions
New Application

AEC Reference No.:	REC104-20
Title:	The influence of rehydration on decomposition in the Highveld Region of South Africa, using a pig model.
Researcher:	Miss CL du Toit
Student's Supervisor:	Dr J Myburgh

Dear Miss CL du Toit,

The **New Application** as supported by documents received between 2020-07-21 and 2020-12-04 for your research, was approved by the Animal Ethics Committee on its quorate meeting of 2020-12-04.

Please note the following about your Conditional ethics approval: The dead pigs must be transported in a closed vehicle in sealed body bags to the burial site.

1. The use of species is approved:

Species	**Number**
Pigs (Sus scrofa)	20 Dead
Samples Pig carcasses (decomposed)	 20

2. Ethics Approval is valid for 1 year and needs to be renewed annually by 2021-12-07.
3. Please remember to use your protocol number (REC104-20) on any documents or correspondence with the AEC regarding your research.
4. Please note that the AEC may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
5. **All incidents** must be reported by the PI by email to Ms Marleze Rheeder (AEC Coordinator) within 3 days, and must be subsequently submitted electronically on the application system within 14 days.
6. As part of your approval, the committee requires that you record a **short video footage** of major animal procedures approved in your study. The committee may request them for monitoring purposes at any later point.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

Room 6-13, Arnold Theiler Building, Onderstepoort
Private Bag X04, Onderstepoort 0110, South Africa
Tel +27 12 529 8434
Fax +27 12 529 8321
Email: marleze.rheeder@up.ac.za

Fakulteit Veeartsenykunde
Lefapha la Diseanse tša Bongakadiruiwa

We wish you the best with your research.
Yours sincerely

Prof V Naidoo
CHAIRMAN: UP-Animal Ethics Committee

Room 6-13, Arnold Theiler Building, Onderstepoort
Private Bag X04, Onderstepoort 0110, South Africa
Tel +27 12 529 8434
Fax +27 12 529 8321
Email: marleze.rheeder@up.ac.za

Fakulteit Veeartsenykunde
Lefapha la Diseanse tša Bongakadiruiwa

Appendix C – Section 20 Approval

agriculture, land reform & rural development

Department:
Agriculture, Land Reform and Rural Development
REPUBLIC OF SOUTH AFRICA

Directorate Animal Health, Department of Agriculture, Land Reform and Rural Development
Private Bag X250, Pretoria 0001
Enquiries: Mr Herry Gololo · Tel: 012 319 7532 · Fax: +27 12 319 7470 E-mail: HerryG@daff.gov.za
Reference: 12/11/1/4/1

Ms. Claire Lynne du Toit/Dr Desiré Brits/Dr Jolandie Myburgh
University of the Witwatersrand
7 York Road
Parktown
Johannesburg
2193

RE: Permission to do research in terms of Section 20 of the ANIMAL DISEASES ACT, 1984 (ACT NO. 35 OF 1984)

Dear researchers

Your fax / memo / letter/ Email dated 24 June 2020, requesting permission under Section 20 of the Animal Disease Act, 1984 (Act No. 35 of 1984) to perform a research project or study, refers.

I am pleased to inform you that permission is hereby granted to perform the following research/study, with the following conditions:

Conditions:

1. This permission does not relieve the researcher of any responsibility which may be placed on him by any other act of the Republic of South Africa;
2. All potentially infectious material utilised or collected during the study is to be destroyed at the completion of the study. Records must be kept for five years for audit purposes. A dispensation application may be made to the Director Animal Health in the event that any of the above is to be stored or distributed;
3. Only dead pigs, acquired from GHB Farms Rooipoort, Bronkhorstspruit may be used for the study. No outside animals may be sourced without written permission from the Director: Animal

4. Pig carcasses are to be transported as described in your application, kept in a sealed body bag.
5. The decomposing carcases are to be kept in the at Forensic Anthropology Body Farm (FABF) on the Miertjie Le Roux Experimental Farm (Tweefontein 541 JR), University of Pretoria
6. Access to the carcases by other pigs (including feral and wild pigs) and predators must be prevented by the fences in place around the study site.
7. Skeletal remains may be kept for future research and teaching at the University of the Witwatersrand, School of Anatomical Sciences, after proper cleaning and treatment.
8. This Section 20 approval is valid until 31 December 2021.

Title of research/study: The influence of rehydration on decomposition in the Highveld Region of South Africa, using a pig model

Researcher (s): Ms. Claire Lynne du Toit/Dr Desiré Brits/Dr Jolandie Myburgh
Institution: University of the Witwatersrand, Johannesburg
Your Ref./ Project Number: AESC20-05-003
Our ref Number: 12/11/1/1/20/1550(HP)

Kind regards,

Maja.

DR. MPHO MAJA
DIRECTOR OF ANIMAL HEALTH
Date:
2020 -07- 14

SUBJECT: **RE: Permission to do research in terms of Section 20 of the ANIMAL DISEASES ACT, 1984 (ACT NO. 35 of 1984)**

Appendix D – Supplementary Figure

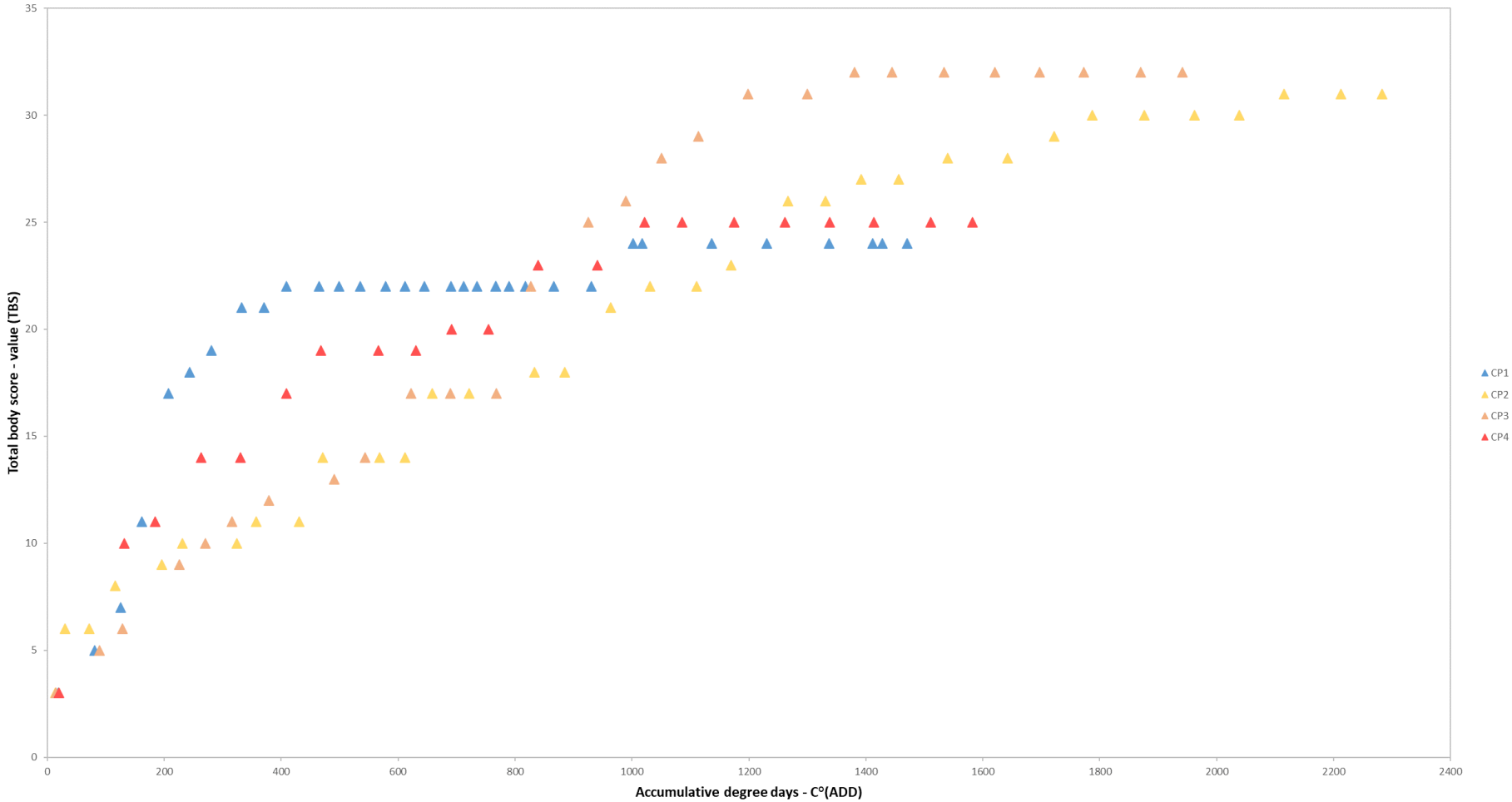


Figure A.1: Comparison of rate of decomposition between all control (triangles) pigs from all four trials by plotting TBS against accumulative degree days (ADD). Colours representing colder (blue) to warmer (yellow, orange, and red) weather conditions throughout experimental phase.

Appendix E – Supplementary Figure

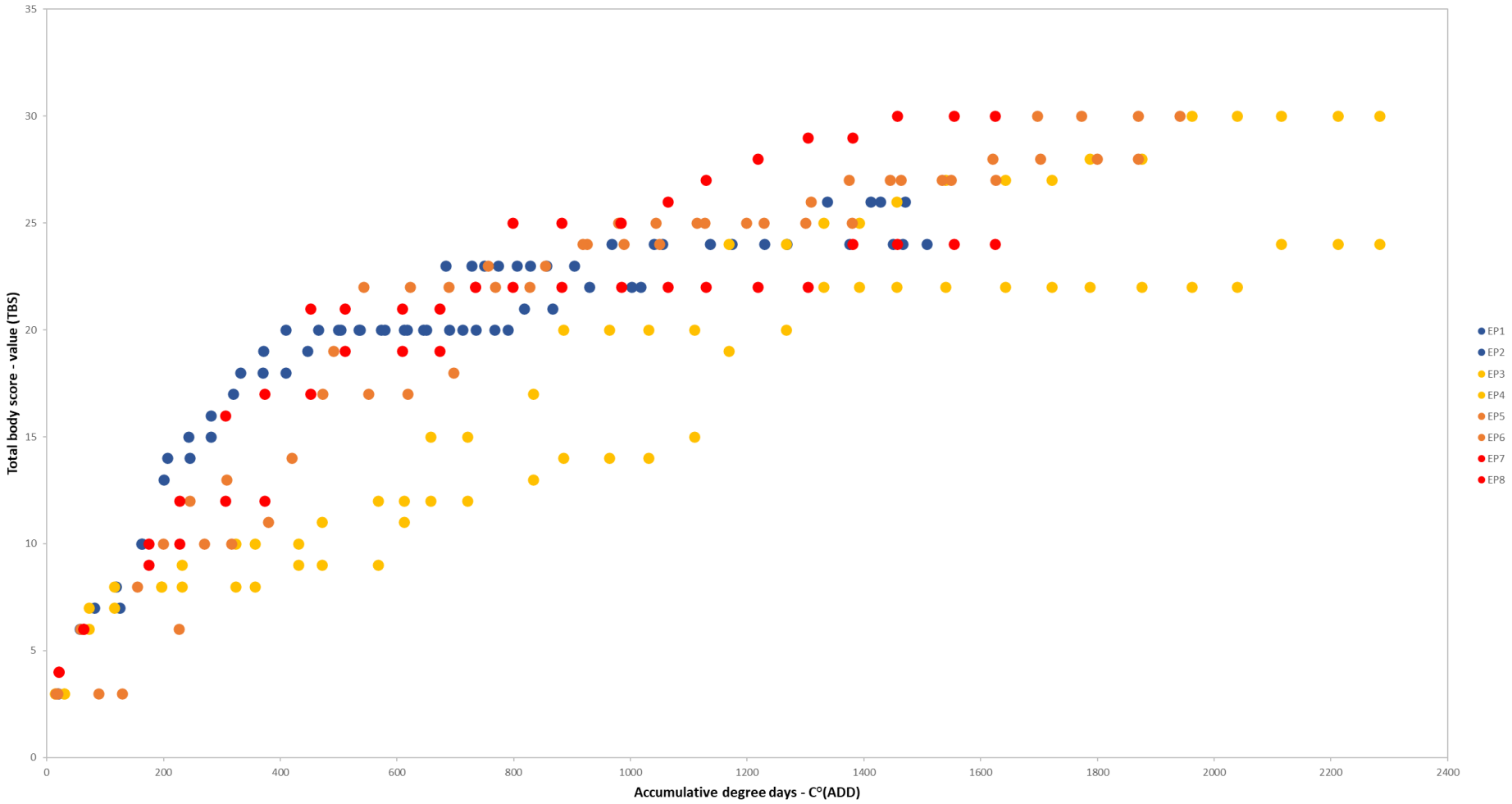


Figure A.2: Comparison of rate of decomposition between all experimental (circles) pigs from all four trials by plotting TBS against accumulative degree days (ADD). Colours representing colder (blue) to warmer (yellow, orange, and red) weather conditions throughout experimental phase.

Appendix F – Repeatability Testing

Intraclass correlation test conducted for CP1

Intra-rater reliability, ICC run on SPSS

Intraclass Correlation Coefficient

	Intraclass Correlation[b]	95% Confidence Interval		F Test with True Value 0		
		Lower Bound	Upper Bound	Value	df1	df2
Single Measures	.991[a]	.981	.996	231.054	27	27
Average Measures	.996[c]	.991	.998	231.054	27	27

Two-way mixed effects model where people effects are random and measures effects are fixed.

a. The estimator is the same, whether the interaction effect is present or not.

b. Type C intraclass correlation coefficients using a consistency definition. The between-measure variance is excluded denominator variance.

c. This estimate is computed assuming the interaction effect is absent, because it is not estimable otherwise.

Inter-rater reliability, ICC run of SPSS

Intraclass Correlation Coefficient

	Intraclass Correlation[b]	95% Confidence Interval		F Test with True Value 0		
		Lower Bound	Upper Bound	Value	df1	df2
Single Measures	.979[a]	.955	.990	94.935	27	27
Average Measures	.989[c]	.977	.995	94.935	27	27

Two-way mixed effects model where people effects are random and measures effects are fixed.

a. The estimator is the same, whether the interaction effect is present or not.

b. Type C intraclass correlation coefficients using a consistency definition. The between-measure variance is excluded denominator variance.

c. This estimate is computed assuming the interaction effect is absent, because it is not estimable otherwise.

Intraclass correlation test conducted for EP1

Intra-rater reliability, ICC run on SPSS

Intraclass Correlation Coefficient

	Intraclass Correlation[b]	95% Confidence Interval		F Test with True Value 0		
		Lower Bound	Upper Bound	Value	df1	df2
Single Measures	.961[a]	.919	.981	50.221	28	28
Average Measures	.980[c]	.958	.991	50.221	28	28

Two-way mixed effects model where people effects are random and measures effects are fixed.

a. The estimator is the same, whether the interaction effect is present or not.

b. Type C intraclass correlation coefficients using a consistency definition. The between-measure variance is excluded denominator variance.

c. This estimate is computed assuming the interaction effect is absent, because it is not estimable otherwise.

Inter-rater reliability, ICC run on SPSS

Intraclass Correlation Coefficient

	Intraclass Correlation[b]	95% Confidence Interval		F Test with True Value 0		
		Lower Bound	Upper Bound	Value	df1	df2
Single Measures	.960[a]	.916	.981	48.425	28	28
Average Measures	.979[c]	.956	.990	48.425	28	28

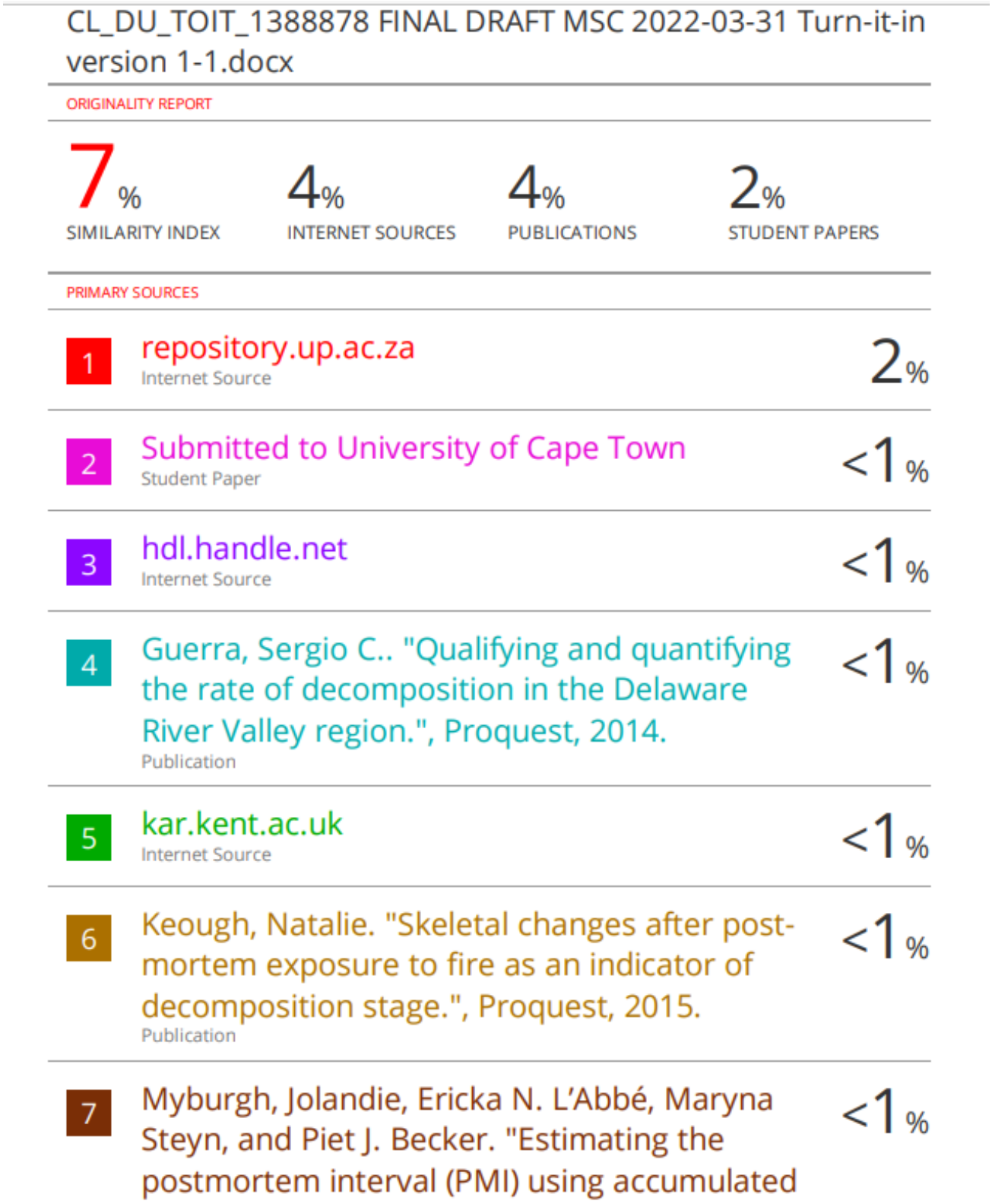
Two-way mixed effects model where people effects are random and measures effects are fixed.

a. The estimator is the same, whether the interaction effect is present or not.

b. Type C intraclass correlation coefficients using a consistency definition. The between-measure variance is excluded denominator variance.

c. This estimate is computed assuming the interaction effect is absent, because it is not estimable otherwise.

Appendix G – Turnitin report



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