THE EFFECT OF SUITCASE CONCELAMENT ON THE INSECT COLONISATION: A PILOT STUDY IN WESTERN AUSTRALIA

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DECLARATION

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

Signed: Christopher Petersen

Jonathon Georgy

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PMI   Post-Mortem Interval

TSD   Time Since Death

TBS   Total Body Score

ADD   Accumulated Degree Days

ABSTRACT

Knowing and understanding the stages involved in the decomposition process of a cadaver is crucial in the ability to accurately estimate the post-mortem interval (PMI) or time since death (TSD). A large amount of information about the PMI can be provided by the state of decomposition as well as the fauna colonizing the body. This is the case if decomposition is treated as a semi-continuous variable and used in conjunction with accumulated degree days (ADD) and by the successional waves of the insects and other arthropods consuming the body. PMI is a pivotal information in forensic investigations as it can be used in homicide cases by personnel of law enforcement for crime scene reconstruction and for the exclusion of potential perpetrators as well as to support witness testimony. Establishing the range of natural events and environmental conditions that were likely to have affected the remains with the passing of seasons can also be aided by knowing the PMI, permitting a more thorough taphonomic analysis.

However, the use of a method to conceal a body (such as burials, wrapping, suitcases) may affect changes within a body and the associated entomological activity that are different to bodies that are exposed to the environment. Therefore, it is necessary to understand the unique taphonomic processes that occur when a body is concealed. While numerous types of concealment have been researched and investigated in the past, minimal research has been specifically related to concealment within a suitcase. This literature review aims to address the effects of different concealment methods, with main focus on suitcase concealment, on the decomposition rate and entomological activity of a cadaver.

KEYWORDS: forensic science, forensic entomology, taphonomy, decomposition, accumulated degree days, post-mortem interval, concealment

1.0   INTRODUCTION

Decomposition is a complex and continuous process that involves the breakdown of soft tissues post mortem. This eventually leads to partial or complete skeletonisation (Goff, 2009; Comstock, 2014). There are series of stages in which the decomposition process has been divided into, with each stage characterised by specific changes in tissue morphology and insect activity (Goff, 2009; Terneny, 2014). Depending on the geographic region and the author, the number of stages has varied from one to as many as nine (Goff, 2009). The decomposition process is often broadly categorised into five stages (fresh, bloated, active decay, advanced decay, and dry/skeleton) despite this varying difference in the number of stages reported (Comstock et al., 2015; Teo et al., 2014). Although researchers frequently use decomposition stages, it is important to understand that in reality, the process of decay doesn't occur in discrete stages (as it is a continuous process) (Comstock, 2015). However, establishing standardised decomposition stages aids criminal investigations in estimating the post-mortem interval (PMI), as well as to assist in describing the decomposition process (Comstock, 2014; Comstock, 2015). In addition, decomposition in court proceedings and research reports can be more easily explained via the use of stages, and it allows the physical appearance of carcasses to be more concisely described by a general term (i.e. the given decomposition stage) (Comstock, 2015).

In an effort to more precisely describe decomposition, scoring systems (a process to describe decomposition quantitatively) have also been established to help divide decomposition into four broad categories (fresh, early decomposition, advanced decomposition, and skeletonisation) (Comstock, 2015). This is particularly useful when differential decomposition occurs. Specific observations of the head and neck, trunk, and limbs are assigned point values in order for the state of decomposition in these areas to be better assessed (Comstock, 2015; Megyesi, Nawrocki, & Haskell, 2005). The process may still be separated into stages however. The sum of the scores (also known as total body score (TBS)) therefore allows the decomposition process to be characterised via a more qualitative system (Comstock, 2015; Megyesi, Nawrocki, & Haskell, 2005).

Even though the basic sequence of decay is generally the same for all carcasses, the rate at which decomposition occurs varies between bodies as a result of many interrelated variables (Anderson, 2016; Mann, Bass, & Meadows, 1990). Both intrinsic (variables concerning the corpse itself) and extrinsic factors (variables in the external environment) may directly

influence the rate of decomposition, with a number of these factors having been previously investigated (Comstock, 2015; Teo et al., 2014; Anderson, 2016; Campobasso, Di Vella, & Introna, 2001). Intrinsic factors include body size, condition of body, health of the individual, cause of death, and presence of clothing, whereas examples of extrinsic factors include weather/environmental conditions such as temperature, humidity, precipitation; as well as the properties of soil, insect accessibility and animal predation (Comstock, 2015; Anderson, 2016; Mann, Bass, & Meadows, 1990; Campobasso, Di Vella, & Introna, 2001; Matuszewski et al., 2014; Sutherland et al., 2014; Payne, 1965; Haslam & Tibbett, 2009; Tumer et al., 2013; Gill, 2015; Anderson, 2001; O'Brien et al., 2007).

Of these many variables, insect activity has shown to be one of the most important when it comes to decomposition rate (Comstock, 2015; Hayman & Oxenham, 2016). Due to their prominent role, insects are frequently included when characterising decomposition stages. The post-mortem interval can also be estimated using entomological evidence (Goff, 1992). However, the role of insects in the decomposition process has been detailed in a number of publications in which bodies are readily accessible. Few have investigated barriers that may affect the access of bodies to insects, and therefore decomposition (Goff, 1992). These include different concealment methods such as burials, being indoors, wrapping a body (eg. blanket), and concealment within a suitcase (Gunn & Bird, 2011; Goff, 1992; Bhadra, Hart, & Hall, 2014).

Changes within the body and the associated entomological activity are affected by each type of concealment in different ways (Bhadra, Hart, & Hall, 2014). This literature review aims to address this scientific area, with the purpose of experimental validation. Without understanding these unique taphonomic processes that occur when a body is concealed, a precise estimation of the duration of time an individual has been deceased will never be obtained. As the occurrence of these methods to conceal a body are becoming more common, it is pertinent for this topic to be understood better by forensic scientists. Therefore, the purpose of this literary review is to determine whether the concealment methods (burials, indoors, wrapping and suitcases) will have an effect, if any, on the decomposition rate and entomological activity of cadavers.

2.0   DECOMPOSITION: AN OVERVIEW

This section aims to address the literature that is currently available in regards to the decomposition rate and insect activity of cadavers. This includes methods to measure decomposition, factors affecting decomposition rate and use of forensic entomology in determining the post mortem interval (PMI). This section will finish by discussing different methods of concealment and their effects on entomological activity, in particular, the succession pattern and species composition of insects.

2.1   MAMMALIAN POST-MORTEM DECOMPOSITION

Generally, decomposition commences after the heart is ceased (Goff, 2009) and is the process by which an organisms soft tissue is degraded until it reaches skeletonisation (Pinheiro, 2006). The tissue can be removed chemically by microorganisms, or physically, by maggots or scavengers (Benninger, Cater, & Forbes, 2008; Goff 2009; Janaway, Percival, & Wilson, 2009; O'Brien, Forbes, Meyer, & Dadour, 2007). The decomposition process involves two parallel processes of putrefaction and autolysis (Benninger et al, 2008; Vass et al., 2002).

Autolysis begins when there is a drop in oxygen levels in the blood and begins destroying cells and tissues of the body by intracellular and extracellular hydrolytic enzymes (Gill-King, 1997; Janaway et al., 2009; Vass et al., 2002). The body shows signs of fluid blisters and skin slippage from the cells being destroyed (Vass et al., 2002). During the destruction of cells, the cellular matter aids the putrefaction process (Janaway et al,. 2009).

Putrefaction is a bacteria driven process that destroys the body's tissue (Campobasso, Di Vella, & Introna, 2001: Janaway et al,. 2009; Vass et al., 2002). These broken down tissues are converted into carbon dioxide, hydrogen sulphide and methane gases by the fermentation of bacteria, with addition of liquids and simple molecules (Gill-King, 1997; Vass et al., 2002). A green discolouration of the skin due to a change in blood (haemoglobin to sulfhaemoglobin), is the first sign of putrefaction (Gill-King, 1997; Vass et al., 2002). The gases listed above, aid in the transportation of sulfhaemoglobin and display a marble-like appearance throughout the body (Pinheiro, 2006).

2.1.1 Characterising Decomposition Stages

Decomposition is broken down into stages in order to describe the decomposition process, in addition, to develop an estimated post-mortem interval (PMI) (Comstock, 2014). Decomposition is categorised into stages, and the number of stages varying from author to author. In 1894, Megnin published the first set of stages on the process of decomposition from an entomological point of view. He reported eight stages, which described the minimum time to reach each stage, the carcasses physical condition, and the type of insects that were present around the carcasses (Megnin, 1894). Observations were made that insects were either attracted or repelled by the type of remains (Johnston & Villeneuve, 1897).

Bornemissza (1956) thought that the climate, season and types of soil are the main influential factors on the decomposition rate and must be taken into account (Bornemissza, 1956). His study was conducted in Perth, Australia, and used the following five stages to outline the decomposition process: initial decay, putrefaction, black putrefaction, butyric putrefaction, and dry decay (Bornemissza, 1956). Payne (1965) used six stages (fresh, bloat, active decay, advanced decay, dry, and remains) based on observations of the carcasses odour, appearance, and insect activity (Payne, 1965). Researchers in Spain examined cases to determine the PMI (Prieto, Magana, & Ubelaker, 2004). They described the decomposition process (putrefaction, early, advanced, and complete skeletonisation), and the preservation of cadavers (mummification and saponification) (Prieto et al., 2004). They discovered the earliest phase allowed a PMI of eight days to be determined. Payne (1965) also created new stages (fresh, bloating and decomposition, flaccidity and dehydration, mummy stage, and desiccation and disintegration) that excluded insect activity. According to Comstock (2014), this is the only study that excludes insects as authors find it difficult to accomplish. Research is yet to be conducted regarding this matter.

2.1.2 Commonly Used Decomposition Stages

The first stage in the process of decomposition is the fresh stage, and begins once the heart stop beating (Goff, 2009). The stopped beating heart will allow blood to flow to the lower parts of the body due to gravity, starting to form discolourations and red areas throughout the body, also known as livor mortis (Goff, 2009; Janaways et al., 2009; Vass et al., 2002). Livor mortis usually begins within an hour of death and is eight hours in length (Baden and Hennessee, 1989). PMI and the positioning of the body can be determined if the body is moved after this time period, as lividity will be fixed and there will be stains at the original site of contact (Perper, 1993).

The second process, rigor mortis, is a build up of lactic acid in the tissues and causes muscle stiffening, and eventually the muscles will become fixed and immobile (Janaway, 1996; Spitz and Fischer, 1980). Rigor mortis occurs approximately two to four hours after death, peaks at twelve hours, then begins to slow down after the 24 hour period, and finally ends by the 36th hour (Janaway, 1996). The body will become limp and flexible, and medical examiners can take advantage of this stage to determine the time since death.

The third process, algor mortis, lowers the body's temperature after death. On average, within the first twelve hours of death a body's temperature decreases by two degrees, and then at a rate of one degree per hour (Perper, 1993). However, factors such as entomological activity and ambient temperature can affect these rates. Calculations can then be made to determine the time since death, to be accurate within a few hours.

At this point, oxygen levels will become depleted and anaerobic microorganisms will proliferate, promoting autolysis (Clark & Worrell, 1997). These microorganisms will convert proteins, lipids and carbohydrates into organic acids and gases (Clark & Worrell, 1997; Gill-King, 1997). The first carrion insects to arrive and seek sites, in opened wounds or natural orifices, to lay eggs and feed are blowflies and flesh flies (Goff, 2009; Payne, 1965).

Putrefaction (anaerobic metabolism) occurs during bloat. This causes a build up of gases and distension of the abdomen which gives the cadaver a bloating appearance (Benninger et al., 2008; Goff, 2009; Payne, 1965). Fluids are forced out of natural orifices (the nose, mouth, and anus), as the gas pressure increase in the body (Carter & Tibbett, 2008).

At this time, maggots will be feeding on the tissue of the cadaver (Payne, 1965). The maggot activity will cause skin slippage and hair loss and eventually lead to post-mortem ruptures of the skin, which purges gases and fluids out of the cadaver resulting in a strong odour (Goff, 2009; Janaway et al., 2009; Payne, 1965).

The active decay stage goes through the most amount of mass loss, from the extensive maggot feeding and the purging of fluids (Carter & Tibbett, 2008; Michaud & Moreau, 2010). Liquefaction and disintegration of tissues and strong odours become evident (Goff, 2009; Payne, 1965). When the maggots migrate away from the body to pupate, that indicates the end of the active decay stage (Goff, 2009).

During advanced decay, insect activity is reduced due to the great loss of biological matter for them to feed on, resulting in nearly a full decomposition inhibition (Carter & Tibbett, 2008). Dead vegetation and the surrounding area of the carcass will show an increase in nutrients (magnesium, calcium, potassium and phosphorus) and an increase in carbon and nitrogen in the soil (Vass, Bass, Wolt, Foss, & Ammons, 1992).

Dry stage begins when there is revival of plant growth around the cadaver due to the excessive nutrient filled soil (Carter & Tibbett, 2008). At this stage, dry skin, cartilage, and bones is all that will remain of the cadaver (Michaud & Moreau, 2010; Payne, 1965). The only distinguishing features in order to identify the start of the dry stage are from the hair, skin, bones, and teeth of the cadaver, which will be bleached and dry (Janaway et al., 2009; Payne, 1965).

Defining each decomposition stage vary from author to author and cannot always be accurately described, due to a variety in conditions at the time. Influential condition can change the process and rate of decomposition, and cause complications in differentiating the stages. Payne (1965) looked at the absence of insects during decomposition of a carcass. This type of study occurs when there is an inhibition factor, such as an enclosed body in a container or a suitcase (Rush, Findlay, & Casey, 2012). This example prolongs the decomposition rate as it limits or inhibits insect activity completely. Other factors that slow down the decomposition rate are cold seasons, high altitudes or when bodies are wrapped or covered with material (Goff, 1992; Kleiss, 2008; Cooper, 2010).

Table 1: Common decomposition stages, features, and visual references adapted from Comstock (2014). Intervals adapted from Clark, Pless and Worrell (1997).

Stage	Characteristic Features	Visual Reference	Intervals
Fresh	Visible changes are minimal Livor, rigor, and algor mortis occur		From zero to seven days
Bloated	Gases accumulate in the abdomen Marbling and skin discolourations are visible Purging of fluids from natural openings		Initiated within the first 48 hours of death and ends by the seventh day
Active Decay	Insect activity is prominent Strong odours Leathery skin		Between the second and eighth day after death
Advanced Decay	Soft tissue remains are minimal Skin is rigidity, thick and discoloured		Initiates after the first week of death
Dry/Skeleton	Minimal skin, bones, and teeth remain		Weeks to months after death

2.2   INHIBITORY EFFECTS ON DECOMPOSITION

In natural conditions, the chemical process of decomposition and dissolution of soft tissue can be stopped by environmental conditions (saponification and mummification) (Forbes and Carter,   2016).

2.2.1 Saponification

Saponification is resulted from the hydrogenation and hydrolysis of adipose tissues, in which adipocere is formed (Pinheiro, 2006). Adipocere is a white-yellowish wax-like substance. When it is bound with interstitial fluids (sodium), it forms a hard composition. When it is bound with potassium from broken down cells, a paste-like complex is formed (Vass, 2001). Factors such as temperature, ventilatioin, moisture, environment, and clothing influence the formation of adipocere (Bardale, 2011). The formation can be in small areas or over the entire body (Forbes and Carter, 2016). Once the formation has been set, the remains can be preserved for a lengthy period of time (Dent, Forbes and Stuart, 2004). It is thought that an altered microbial environment with reduced pH levels is the key reason for tissue preservation (Forbes and Carter, 2016). Figure 2.1 illustrates an overview of the saponification process.
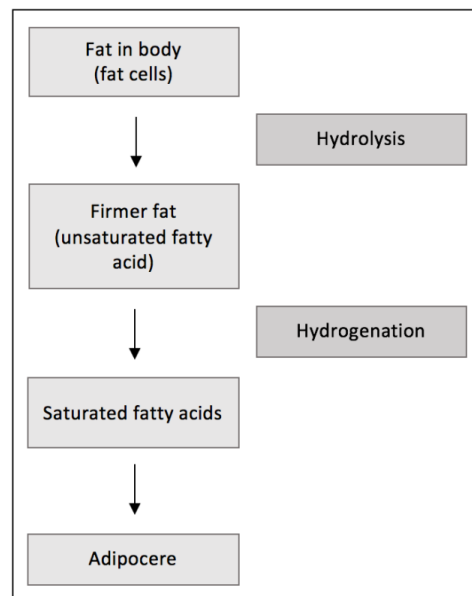
Figure 1: Overview of saponification.

Source: Bardale, 2011.

2.2.2 Mummification

Mummification is the process of conservation formed by dehydrated tissues (Pinheiro, 2006; Vass, 2001). Soft tissues begin to decompose beneath the dry, hardened and leathery skin that remains on the bone (Dix and Calaluce, 1999). Mummification may only be present in specific areas or observed throughout the entire body (Forbes and Carter, 2016). The formation process is dependent on conditions such as ventilation, the physical size of the cadaver and environment conditions (Bardale, 2011). Favourable conditions for the formation are hot, dry and low humidity environments (Vass, 2001; Dix and Calaluce, 1999). In these extreme conditions, bacterial and insect activity is slowed down as the soft tissue is dehydrated and the body may be preserved for years (Forbes and Carter, 2016; Dix and Calaluce, 1999). Figure 2.2 illustrates an overview of the mummification process.
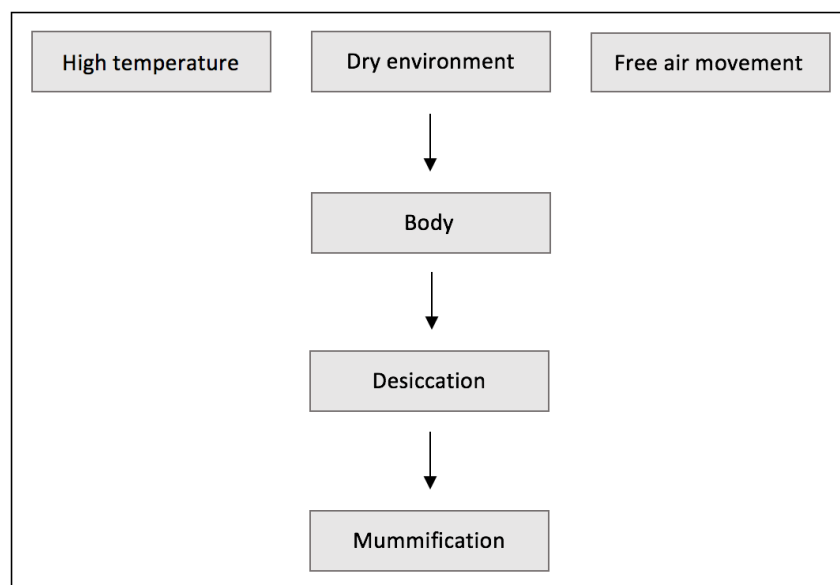

Figure 2: Overview of mummification

Source: Bardale 2011.

2.3   MEASURING DECOMPOSITION

Several small changes occur throughout the sequential process of soft tissue decomposition (Megyesi, Nawrocki, & Haskell, 2005). If the extensive, qualitative classes of decomposition were modernised to particularly mirror those small sequential changes, the condition of decomposition can be reported more accurately and precisely, as a continuous or quasi-nonstop variable (Megyesi, Nawrocki, & Haskell, 2005). Therefore, more information about the relationship between decomposition and the PMI could be provided via the usage of an extra range of quantified stages with assigned point values to convey decomposition, in place of the use of a small number of qualitative categories of decomposition (Megyesi, Nawrocki, & Haskell, 2005). The statistical power of hypothesis testing could also be increased (Megyesi, Nawrocki, & Haskell, 2005).

Megyesi and colleagues proposed such a method in 2005 known as the total body score (TBS). This scoring method firstly divides decomposition into four broad categories. These include fresh, early decomposition, advanced decomposition, and skeletonisation. Each category is further subdivided into a number of stages, which describe the basic characteristics and appearance of the remains. Each stage is then assigned a point value, starting at 1 for fresh and increasing one point for each progressive stage. However, based on their composition, various parts of the body decompose at unequal rates (Megyesi, Nawrocki, & Haskell, 2005). To account for this differential decomposition, Megyesi (2005) divided the body into three specific areas ((1) head and neck, (2) trunk, (3) limbs) and scored each independently.

The final TBS is then produced by combining the scores of each of the three anatomical regions. Megyesi (2005) provides an example using case X. Hypothetically, if all three regions were classified as being in the early stage of decomposition in the fourth category, this would result in a TBS score of 5+5+5=15. If a subject was to appear fresh in all regions, the lowest score it could receive is a 3. The highest score it could receive would be 35 (dry and fully skeletonised in all regions). Megysei et al., (2005) also noted in some cases across one anatomical area that the decomposition stage varied (eg. decomposition of arm and leg did not match). In these instances, the average point value of the two extremes observed in the area was assigned.

Figure 3: Categories and stages for each of the anatomical regions along with assigned point values. Source: Megyesi, Nawrocki, & Haskell, 2005.

Using varying methodologies, numerous decomposition studies have been carried out in different climatic conditions and seasons (Myburgh et al., 2013). Depending on the environment, the rate of decomposition will vary. Hence, for specific geographic regions within each country, PMI estimates need to be developed (Myburgh et al., 2013). This makes the accumulated degree days (ADD) model appropriate for comparison. ADD are defined as heat energy units that are available to drive reactions (both chemical and biological) to take place in soft tissue decomposition (Myburgh et al., 2013). For this reason, both temperature and chronological time combined are represented by ADD. Therefore, the effect of temperature in multiple regions is eliminated by making use of ADD, and allows comparisons between different studies to be made (Myburgh et al., 2013).

In addition to the TBS method, Megyesi et al., (2005) made use of accumulated degree days (ADD) to quantitatively estimate the post-mortem interval. In order to establish an accurate PMI and estimate the decomposition rate, Carter et al. 2007 believes combining the method of accumulated degree days with the scoring method for decomposition may be the most accurate process. To calculate ADD, Megyesi (2005) collected temperature data (maximum and minimum air temperature) each day from the nearest National Weather Service Station and presented it in the form of daily averages (average of both minimum maximum values). All daily average temperature values were added together for all days from death until discovery to give a final ADD value. After completion of the data analysis using linear regression, Megyesi et al. (2005) mentioned that ADD was the cause of approximately 80% of the variation in the decomposition process. Therefore, rather than just solely modelling decomposition as being dependent on elapsed time since death, they suggested that accumulated temperature should also be taken into account. The global implemention of time since death estimations may be assisted by methods that use ADD (Megyesi, Nawrocki, & Haskell, 2005). This is the case as time scales for the post-mortem interval can be adjusted via the use and knowledge of temperature. Using ADD data has allowed a number of studies to measure the effects of other variables on decomposition rate (such as submersion and burial), due to the elimination of temperature effects (Megyesi, Nawrocki, & Haskell, 2005).

Several studies since Megyesi (2005) have utilised the TBS method, either with or without modification. In addition, the accuracy and overall value of the ADD/TBS scoring method developed by Megyesi (2005) has been assessed by numerous studies. However, it came to the attention of Dabbs et al. (2016) that no studies had examined the reliability of the scoring method. Thus, while the accuracy of predicting PMI (given a specific ADD) via the regression equation has been examined by several authors, it is still unknown whether different users apply the method for quantifying decomposition consistently at different times and places (Dabbs, Connor, & Bytheway, 2016). This was investigated by Dabbs et al. (2016) and conducted by testing interobserver error rates. Overall, Dabbs et al. (2016) concluded that the TBS method for quantifying decomposition had low levels of interobserver error (high reliability) with the consistency between the observers in this study being "near perfect". Their null hypothesis that different observers would produce a difference in the scores was rejected as during the course of the research, none of the differences observed (education, experience, individual variability) were statistically significant ($p < 0.001$ for all

comparisons). Therefore, in using TBS for PMI estimation, the scoring method for TBS was eliminated as a potential source of error (Dabbs, Connor, & Bytheway, 2016).

In order to further improve reliability of the TBS system, Dabbs et al. (2016) suggested some slight changes; incorporating the word "natural" instead of "pink" when referring to flesh colour; double-checking the data on a regular basis for simple errors (eg. using limbs table for trunk); each quadrant of the trunk as well as arms and legs to be scored separately and then averaging the scores; the scoring system to include a range of colour changes; and the bloating of limbs to be included. However these need to be tested and evaluated first before implementation to see if observer error is in fact reduced.

2.4   FACTORS THAT INFLUENCE RATE OF DECAY

Generally speaking, variables may influence that rate of decay by speeding up or slowing down the decomposition process, however, the stages of decomposition are still the same (Janaway et al., 2009). The rate is influenced by extrinsic and intrinsic factors (Campobasso et al., 2001). The most common variables are temperature, moisture, insect activity, and sun or shade exposure (Dautartas, 2009). In addition, if the remains have been buried or let on the surface, this will also be influenced. These influences are visible throughout the decomposition process.

2.4.1 Temperature

Generally speaking, warmer conditions will accelerate the decay process, and cooler climates will delay or stop the process (Smith, 1984). Warmer conditions promote bacterial activity which assists in decomposition. In addition, plant and vegetation surrounding the area may affect decomposition and temperature. Mant (1987) discovered that pine needles and straw retained heat when covering decomposing matter. The cold temperature has a preservation effect on the body's tissue and often inhibits insect and scavenger activity (Janaway, 1996).

2.4.2 Moisture

A moister environment advances decomposition, and a dryer climate delays the process (Smith, 1984). A dry environment is shown to be favourable to the mummification stage of decomposition (Mant, 1987). A combination of dryness and heat is an example of how more than one factor can interact and greatly influence the decomposition rate; it is uncommon for

a single factor alone to determine the decomposition rate (Mant, 1987). A rat carcass was used to observe the effect of moisture on the rate of decomposition by Aturaliya and Lukasewyz (1999). They discovered that the most significant impact in the loss of moisture was from material or environment that was directly in contact with the decomposing bodies skin (Aturaliya and Lukasewyz, 1999). Materials that helped absorb moisture led to a faster mummification process. On the other hand, if materials collected water mummification was prevented, in spite of the body being buried or on the surface (Aturaliya and Lukasewyz, 1999).

2.4.3 Individual Variation

Physical conditions and structure of a body can affect decomposition (Stuart, 2003). A body that consists of higher body fat will generally take longer to skeletonise, than a thinner body, this is due to a bigger body can delay heat, and heat is essential for a faster decomposition rate (Gonzales et al, 1954; Mant, 1987). In addition, bacteria growth needs a sufficient amount of liquid, which is active throughout the stages of decomposition. Although bodies with more body fat commence decomposition quicker, the overall process to skeletonisation requires more time. Unnatural wounds (gunshots or stabbings) also speeds up the rate of decomposition, due to insects being more attracted to the additional exposures on the body (Mann et al, 1990).

2.4.4 Oxygen Content

Access or restricted oxygen to the body is another influential factor (Mant, 1987). Putrefaction from aerobic bacterial activity is a significant part, and oxygen is required to fuel the process. Mant (1987) compared an exposed body that had a wound, with a buried body (not exposed to air). Mant (1987) discovered that the exposed body had signs of advanced decomposition, showing great loss of soft tissue.

2.4.5 Insects

Insect activity accelerates the decomposition process (Gonzales et al., 1954), and is debatably the most influential accelerant. Blowflies are typically observed on a corpse minutes after deposition, and are the generally the first insects to be attracted (Campobasso et al., 2001). They lay eggs around the natural orifices or any open wounds, and between eight to fourteen hours, maggots are hatched (Campobasso et al., 2001).

Payne (1965) observed the comparison of decomposing pigs exposed and not exposed to insects. The colonised pigs had a faster decomposing rate than the pigs that were absent of insects. The pig carcass would need available bacteria for the insects to accelerate the disintegration and liquefaction process. A more recent study by Simmons et al (2010) concluded from studies that the effects of insect exclusion slowed down a significant amount if insects were not colonising on carcasses (Simmons, Cross, Adlam, & Moffatt, 2010).

2.4.5.1 Maggot Activity

With a large number of maggots being produced and soft tissue being consumed within a short period of time, this leads to liquefaction of the body quickly (Evans, 1963). While maggots travel and feed around the body, they are producing a great amount of heat and bacteria distribution, which help speed up the rate of decomposition (Lord, 1990; Mann et al., 1990). In saying this, insect activity does depend on ambient temperature, and on sun or shade exposure. Sunlight speeds up the process and shaded areas slow down the process (Srnka, 2003).

2.4.5.2 Seasonality

Certain insects will only appear during the warmer seasons, while others appear over the cooler weather (Greenberg and Kunich, 2002). This information assists determining the PMI and possible movement of the body. Seasonality data must be used carefully, due to the fact that bodies can be indoors with imitation temperature settings could be used to mislead insects and seasonality evidence, as well as abnormal weather conditions, such as heat waves (Greenberg and Kunich, 2002).

2.4.5.3 Secondary Burial and Insect Activity

If a body was placed in a grave a number of days after death, evidence of different life stages of flies would be present and an absence of species that would normally colonise a body in the later decomposition stages (Haskell, N.H., Hall, R.D., Cervenka, V.J., & Clark, M.A., 1997). Deep burials can limit and prevent insect access to a corpse (Greenberg and Kunich, 2002). If insect activity is present from a corpse that was buried, this suggests that the body was exposed to surface conditions prior to the burial.

2.4.6 Activity of the Macro-Fauna

Mammalian carnivores and rodents can contribute to the rate of decomposition. Larger carnivores (wolves, dogs or foxes) are usually the primary responsible for eating areas such as the face, neck, and abdominal areas (Willey and Snyder, 1989). Rodents focus their eating habits on the long bones, opposed to soft tissues (Haglund et al., 1989; Klippel and Synstelien, 2007). Both rodents and mammalian carnivores are known to scatter and leave remains behind.

2.4.6.1 Surface vs. Buried

Buried remains prevent insects and mammalian scavenging activities, therefore slowing down the decay process. The temperature below the ground is a lot cooler than above the surface, which will also affect the rate and slow it down (Janaway, 1996). In addition, damaging weather conditions to the tissue of the body will also be prevented than a body on the surface. The decomposition rate of the body will vary depending on the depth of the burial. The deeper the burial is, the better preservation occurs, due to a more stable environment and protection (Mant, 1987).

Buried bodies are more difficult to detect, which can prolong the process of decomposition before being found and analysed. Vass et al. (2004) are researching a chemical sensor to detect the odours that are released from buried bodies, without the aid of cadaver dogs.

2.4.7 Clothing

Clothing promotes the formation of adipocere, which is an insolulble soap formed from fatty acids that hydrolyse with bivalent ions (Jackowski et al., 2005; Miller, 2002). Depending on certain factors, clothing has shown to both accelerate and slow decomposition (Cahoon, 1992; Miller, 2002).

2.4.7.1 Other Coverings

Although clothing is the most commonly material covering a body, there have been cases in New Mexico and Singapore of individuals being wrapped in plastic. It has been reported that the individuals wrapped in plastic were in a state of much higher preservation than expected (Chui, 2006). This shows that coverings can significantly affect PMI estimations.

2.5   ESTIMATING THE POST MORTEM INTERVAL

Forensic taphonomy is the study of the changes to remains after death, by observing the factors that influence the decomposition process (Haglund & Sorg, 1997). Obtaining the most accurate estimation of PMI is the primary goal of forensic taphonomy and critical for criminal investigations to help identify suspects and the deceased (Catts, 1992; Haglund & Sorg, 1997). PMI estimations are generally based on opinion and experience by forensic scientists (Vass et al., 2002).

2.5.1 Early Post-Mortem Period

During the early stages of post-mortem, a PMI will be estimated by pathologists from observing the body's changes since death, including livor, rigor, and algor mortis (Amendt et al., 2007; Vass et al., 2002). To determine the most accurate PMI prior to putrefaction, observations in the vitreous humor (the fluid in the posterior chamber of the eye) for biochemicals signs such as potassium ion and hypoxanthine concentrations, will help pathologists (Swann, Forbes, & Lewis, 2010b; Coe, 1993). According to Coe (1993), the vitreous humor is the optimal choice of fluids because it is anatomically confined and confinement reduces putrefaction changes, enzyme activity, and bacterial contamination. In addition, Madea (2005), states autolysis is the slowest in the vitreous humor fluid, allowing up to 120 hours post-mortem to use the fluids concentration gradients. With comparison to blood (can be used up to a few hours after death) and cerebrospinal fluid (between 15-20 hours after death) (Madea, 2005).

These methods can only be applied before the putrefaction period begins (first few days after death). Entomological evidence will take over and be used after the first seven days, and can be used to estimate the PMI from a few weeks up to months (Amendt et al., 2007; Anderson & VanLaerhoven, 1996; Campobasso & Introna, 2001).

2.5.2 Entomological Data

Forensic entomology involves the study of arthropods and insects in criminal investigations to determine the PMI (Amendt et al., 2007; Catts & Goff, 1992). Generally, there are four ecological categories within a carrion community (necrophagous species, that feed on the carrion; predators and parasites, that feed on other insects or arthropods; omnivorous species, that feed on the colonisers and the corpse; and incidentals, which use the corpse as an environment (Campobasso et al., 2001; Catts & Goff, 1992). Flies (*Diptera*) and beetles (*Coleoptera*) are the most dominant in the first two categories (Catts & Goff, 1992; Shean, Messinger, & Papworth, 1993). Flies can detect a carcass from its produced odours, and are usually the first colonisers (Archer & Elgar, 2003; Janaway et al., 2009). They are attracted to carcasses for the purpose of laying eggs in the natural orifices (Archer & Elgar, 2003).

Forensic entomology can aid a criminal investigation by potentially establishing an association between the scene and the suspect. Insects can be used to determine the cause of death, by locating any perimortem wounds (stabbing or gunshot), and a body's original position if it has been moved (Shean et al., 1993). The use of drugs can be determined by using chemical analyses on insects (Campobasso & Introna, 2001). However, estimating the PMI is the most useful application of entomology with a criminal investigation (Catts & Goff, 1992). Knowing the PMI leads to reconstructing the events and circumstance of the scene, establish reliable witness statements, and potentially link up the suspect to the scene (Amendt et al., 2007).

The first wave of insects will be attracted to the carcass when it is moist, with natural orifices, wounds and blood, so they can feed and oviposit (Catts, 1992). After the first wave of insects, different species will be attracted to the carcass depending on the matter and odours throughout the decomposition process, therefore, the sequence of species will be somewhat predictable (Anderson & VanLaerhoven, 1996; Archer & Elgar, 2003).

PMI can be determined from two ways using entomological data. The first method is through the early decomposition, where the estimated PMI is from the time that each species collected from the crime scene to its oldest growth form (Amendt et al., 2007; Catts & Goff, 1992). The second method focuses on the composition of the arthropod activity in the later stages of decomposition, relating to successional patterns, since insects the sequence of species are

predictable depending on the geographic location (Amendt et al., 2007; Anderson & VanLaerhoven, 1996; Catts & Goff, 1992).

2.6   ACCESS OF THE BODY TO INSECTS

A temporary, altering habitat can be provided by decomposing remains to support large arthropod and microorganism communities (Avila & Goff, 1998). A predictable sequence is usually followed soon after death for a body's invasion by insects and arthropods (Voss, Forbes, & Dadour, 2008). Globally, forensic researchers have well documented this succession pattern of arthropods on a cadaver. The post mortem interval (PMI) can then be estimated via the use of arthropod fauna analysis, with knowledge of the arthropod succession pattern on a cadaver (Voss, Forbes, & Dadour, 2008). In forensic investigations worldwide, police services frequently use this aspect of forensic entomology in cases with an unknown time of death (Dadour et al., 2001). In addition, it is essential to give thought to the factors which may have an effect on the patterns and rates of insect invasion on the body, when using arthropods for the estimation of PMI. According to Mann et al. (1990), the second most significant variable affecting decomposition rate is access of the remains to insects. Previous studies have investigated the influence of environmental conditions (such as temperature, humidity and rainfall) (Voss, Forbes, & Dadour, 2008; Goff, 1992). The initial time of arrival of insects on a body may also be affected by the circumstances surrounding the death scenario (Voss, Forbes, & Dadour, 2008; Catts & Goff, 1992). Other studies have also looked at the effect of hanging and burning a carcass on decomposition and, eventually, arthropod succession (Lynch-Aird, Moffatt, & Simmons, 2015).

To a large degree, many case studies that have been previously published involving PMI estimation have detailed methodologies for bodies which have been readily available for insect colonization (such as those being outdoors) Goff, 1992). Much less available studies have investigated situations in which the access of bodies by insects is prevented readily due to barriers such as those found indoors, concealed within a suitcase, wrapped in plastic or cloth, and burials (Goff, 1992).

2.6.1 Concealment

Concealing the body is often a common method in cases of homicide used by perpetrators to evade detection by investigators (Bhadra, Hart, & Hall, 2014). Some common body concealment methods include: shallow grave burials, wrapping the body in blankets or plastic, or even placing the body inside a suitcase before being deposited at a distance from the crime scene. The discovery of bodies by investigators is not only delayed by these types of concealment, but the capability of necrophagous insects to identify and colonise the corpse can also be inhibited (Bhadra, Hart, & Hall, 2014). In the applicability of an approach to entomology, the cadaver's accessibility for insects plays a significant role (Bugelli et al., 2015). Insects do not have direct entry or access to the body when it is concealed. Therefore, errors in the process of PMI estimation can be caused by this inability immediately following death of insects to access the body (Bugelli et al., 2015). This is especially the case if the specific type of concealment has not been taken into account when correct adjustments are to be made. Each type of concealment will have differences in species composition and in time of their arrival on the body (insect colonisation) (Bhadra, Hart, & Hall, 2014; Bugelli et al., 2015).

BURIAL

Burial is a fairly frequent method of concealment used by perpetrators, with shallow clandestine grave burials being the main choice to bury most murder victims (Gunn & Bird, 2011). This is due to the amount of effort and time necessary to dig to any depth as well as the size and weight of the typical adult human (Gunn & Bird, 2011). For a corpse that is discovered above ground, blowflies are typically amongst the first insects to make an appearance (Gunn & Bird, 2011; Pohjoismäki et al., 2010). For buried remains however, hardly any information is available on blowfly colonisation (Gunn & Bird, 2011). Various authors believe that a body being colonised by blowflies can even be prevented by only a slight covering of soil. This is because female flies won't lay their eggs unless physical contact with an appropriate larval food source is obtained (Gunn & Bird, 2011). Despite this, there are numerous accounts where the smell emerging from the ground attracts adult blowflies to the site of a buried body (Rodriguez & Bass, 1985; VanLaerhoven & Anderson, 1999). While blowflies find it difficult to gain access to buried remains, colonising buried remains can certainly be achieved by several fly species such as the adult of the Coffin fly

(*Conicera tibialis)* (Gunn & Bird, 2011). It most likely gains access to the remains due to its size (being very small) and by navigating through the channels and cracks in the soil (Gunn & Bird, 2011). Also from buried remains, the larvae of some muscid genera (*Muscina* and *Ophyra)* have been noted, but the soil overlaying the body is probably the main target for egg laying by adult female flies in these species (Dadour & Harvey, 2008). A host of variables will affect a body's colonisation by Diptera and other invertebrates as well as its decomposition rate, due to all clandestine burials being highly individual events. The geographical location, time of year, the depth of burial, size of the body, and the physical and chemical characteristics of the soil and/or covering material are such variables (Gunn & Bird, 2011). In terms of insect colonisation in burials, Pastula (2013) demonstrated that this can be delayed between 5 and 30 days. This was largely dependent however on the climatic and soil conditions. In contrast, there was no significant difference in insect colonisation and decomposition rate of carcasses that are buried shown by Simmons et al. (2010).

WRAPPING

The time of colonisation as well as the species composition of flies that colonise the cadaver may also be affected by concealing a body by some sort of wrapping. According to Ahmad et al. (2011), blowfly colonisation can be delayed by 24 hours or more due to the accessibility to suitable oviposition sites being blocked by the wrapping of a cadaver. It was also concluded in this study that there were differences in the abundance of fly species present in carcasses wrapped in an empty rice sack material compared to those not wrapped. In wrapped carcasses, families such as Calliphoridae, Muscidae, and Phoridae were more abundant with Piophilidae, Sepsidae, and Psychodidae being more prevalent in exposed carcasses (Ahmad et al., 2011). However, species composition was not affected by wrapping; only the occurrence of some species was prolonged (Ahmad et al., 2011). All families collected were encountered in both wrapping and exposed carcasses. In a study performed by Goff (1992) (which was unreplicated), wrapping pig cadavers in heavy blankets resulted in the fly attendance being delayed 2.5 days. Voss et al. (2008) was also in agreement with Goff that the invasion of a corpse by blowflies can be delayed for up to several days by using multiple layers of blanket to wrap a body.

INDOORS

It has been shown in many articles previously published that in remains concealed indoors, insect colonisation is delayed in comparison to those situated outdoors (Cammack et al., 2016; Bugelli et al., 2015; Pohjoismäki et al., 2010; Charabidze, Hedouin & Gosset, 2015). In addition, different insect species have been reported to colonise remains as well as differences in pattern succession from indoor and outdoor conditions (Bugelli et al., 2015). For cadavers exposed in the field, Bugelli et al. (2005) has described the scuttle flies (Diptera, Phoridae) as being later colonisers. However in cases where cadavers are found indoors, the scuttle flies are among the first colonisers. Possible bias affecting the estimation of PMI in indoor forensic entomology was considered by Reibe and Madea (2009). In this particular experiment, the insect colonisation of piglets was delayed and less frequent when positioned inside a room with a slightly opened window when compared to piglets placed in an outdoor location approximately 50 metres away (Reibe & Madea, 2009). Similar results were also obtained by Anderson (2011), in which indoor and outdoor environments were utilised for the investigation of decomposition rates; the colonisation of corpses located outdoors occurred 5 days later than the outdoors located corpses, and fewer insects were observed. Finally, an article published by Pohjoismäki et al. (2010) in which nine indoor forensic entomology cases were examined, highlighted similar issues. Pohjoismäki et al. (2010) also mentioned that the building's structure and composition must also be taken into account as this can affect both access to corpses and the diffusion of odours.

SUITCASE

During recent years, the use of suitcases as a mode to conceal bodies has become more common, with the most recent case in Western Australia occurring in 2016 where the body of Annabelle Chen was found in a suitcase in the Swan River. There are a few likely reasons behind this; not only are suitcases useful for concealing a body, but they also allow the body to be easily transported, and therefore reduce the likelihood of detection during both transport and disposal. The decomposition of a cadaver in a suitcase however is one scenario which has not been well documented in the past. While there has been research into numerous types of concealment, there has been minimal research specifically related to concealment within a suitcase. To this date, no research on suitcase concealment of a body has been performed in Australia. The only known research has occurred in the United Kingdom; Bhadra et al.,

(2014) investigated the use of suitcases and the accessibility of this type of concealment to blowflies. In addition, the prospect of oviposition and infestation under these circumstances was also scrutinised. Only after 48 hours were flies first seen to be attracted to the suitcases containing pig heads, with oviposition first being noticed at 72 hours (Bhadra, Hart, & Hall, 2014). It was also mentioned that a primary factor which may influence fly oviposition on suitcases containing bodies was the zip fastener used on the suitcase, due to this being the most likely point of entry (Bhadra, Hart, & Hall, 2014). Bhadra et al., (2014) believes however that conclusions can't be drawn from this study alone (due to the minimal research) and that further research needs to take place. This is the reason why this particular study has been conducted.

3.0   EXPERIMENTAL DESIGN, SAMPLING AND ANALYSIS

Six medium-sized (approximately 7-11 kg) pig carcasses will be utilised for the research objectives over a six week period (April - May). One pig carcass will be used as the controlled variable, lying on its side in a shaded aviary cage shelter without any materials covering the pig carcass. The other five pig carcasses will each be enclosed in identical suitcases (placed at least 15 metres apart from each other) in a shaded area. The first suitcase will be opened after two weeks (14 days), with a seven day interval between the rest of the suitcases. For example, suitcase one will be opened on the 14th day; suitcase two will be opened on the 21st day; suitcase three will be opened on the 28th day, and so on, until the end of the six week period (42 days). Payne (1965) states that maggots will be present in the pigs natural orifices and will feed throughout the earlier stages of decomposition. To provide a focal point for sample collections, each pig will have sticky traps surrounding the area, and samples of insects will be collected at random from the orifices of the controlled pig. The suitcases will be examined carefully for any laid eggs and maggots, and the focal point will be around the zippers.

When examining the stages and rates of decomposition, taking into considerations of the temperature and insect activity is critical. During the decomposition process, different insects will be attracted to the pig at different stages depending on the odours released (Comstock, 2014). To reduce the number of variables, the pigs have been placed in suitcases and secured with padlocks to limit scavenging activity. To avoid an increase in temperature, the pigs will be placed in shaded areas. To protect the pigs from scavengers, the pigs have been caged in an aviary (controlled) or in a suitcase (tests).

Once the pig carcasses are in position, observations will be documented and photographed on each experimental day. Observations will be made on the physical appearance by using the TBS method to score the rate of decomposition and any insects present. Samples of insects will be collected and taken back to the laboratory for further analysis. At the laboratory, maturing of the collected eggs and maggots into adult stages will be observed for identifying purposes. In addition, all other insect samples will be identified.

Decomposition Research Facility

The research will take place at the Murdoch University Veterinary Farm, 90 South St, Murdoch, Western Australia, Australia, 6150. Within the field area is an aviary cage structure that will be used for the exposed pig carcass (control), as it will protect the carcass from unwanted larger scavengers. In addition, the aviary will protect the carcass from any direct sunlight and rainfall. The other five pigs will be enclosed in fabric suitcases placed at least 15 metres apart from each other. The suitcase will be positioned in a shaded area and also act to protect the carcasses from any direct sunlight and possible rainfall. With these extrinsic factors limited, the research can focus more on the affect of insects on an exposed carcass verse a concealed carcass.

Experimental Subjects

Pig carcasses will be used as substitute for human cadavers as they are acceptably similar to human weight, fat to muscle ratio, physiology and biochemistry, and they avoid the ethical issues with the use of human cadavers (Schoenly et al, 2006; rance et al. 1992). The pigs will be euthanized humanly in accordance with animal ethics, and obtained from the Nambeelup Piggery in Mandurah. The pig carcasses will be of similar weight (approx. 10 kg) to decrease variability in results. They will be received fresh, and wrapped in plastic for transportation purposes to the field area.

Weather Data

Weather loggers (six in total) will be placed within the research site and will be set to record the temperature at 30 minute intervals. One logger will be placed in each suitcase, and the remaining logger will be placed next to the control pig carcass. In addition, for comparative purposes, the data from the Murdoch University Weather Station will also be recorded, and the elapsed time will be documented as accumulated degree days (ADD), to account for temperature data. ADD will represent heat energy which maintains the biological and chemical processes that are essential for decomposition. Using ADD allows for decomposition research to be more comparable between environments, seasons, and years, as the temperature data is accounted for (Megyesi, Nawrocki and Haskell, 2005). At the end of the six week research, the data will be transferred to a computer for analysis.

Adaptations to Study Design

The most influential factors affecting the rate of decay include insect activity and temperature. This study design compares a different environmental condition (suitcases), which may limit insect activity, with an exposed pig in an aviary cage (control), to determine more thoroughly how insect activity affects the decomposition rate. Other factors that influence the rate of decomposition consist of wounds to the carcass, the size and weight of the carcass, and the environment (Hau et al., 2014). All these factors will be taken into consideration as the research is designed to have all pigs without wounds, the same size and weight, and similar environments. This can then show how age and wounds can potentially influence the decomposition rate. In addition, different environment conditions such as soil, direct sunlight, and coverings wrapped around the carcass can potentially be used to assess any environmental interference.

4.0   EXPERIMENTAL AIMS AND HYPOTHESIS

In light of the research presented in the literary review, it is evident that each type of concealment (including the use of suitcases) may have an effect on the decomposition rate and entomological activity of cadavers. The main reason behind this may be due to the fact that insects don't have direct access to the body. Subsequently, there are two hypotheses to be tested:

Experimental Hypothesis 1:

H_0:   Concealment of a corpse within a suitcase **will not** have an effect on the rate of decomposition.

H_1:   Concealment of a corpse within a suitcase **will** have an effect on the rate of decomposition.

Experimental Hypothesis 2:

H_0:   Concealment of a corpse within a suitcase **will not** have an effect on the entomological activity.

H_1:   Concealment of a corpse within a suitcase **will** have an effect on the entomological activity.

5.0   CONCLUSION

This review aims to address the literature that is currently available in regards to the decomposition rate and insect activity of cadavers. The state of decomposition of a cadaver can often be relied upon in the estimation of the post-mortem interval (PMI). A large amount of information can be provided by the state of decomposition about the PMI (Megyesi, Nawrocki & Haskell, 2005). With different seasons throughout the year, the range and variety of natural events and environmental conditions that are likely to affect remains can be established by knowing the PMI (Megyesi, Nawrocki, & Haskell, 2005). In an effort to more precisely describe decomposition, scoring systems have also been established (Comstock, 2015). As a result, a more thorough analysis of taphonomy can be permitted. For this reason, it is highly important that forensic investigators are able to identify the PMI from a scene. This is achieved through the use of methods to measure decomposition. Methods such as the five commonly used stages to differentiate decomposition (fresh, bloat, active decay, advanced decay, and dry/skeletal), the TBS method, and the ADD method. Megyesi et al., (2005) made use of ADD to quantitatively estimate the PMI. In order to establish an accurate PMI and decomposition rate, Carter et al. (2007) believes combining the ADD method with the scoring method for decomposition (TBS) may possibly be the most accurate process. The use of these methods have been practiced extensively on exposed bodies, however, on concealed bodies may affect changes associated with entomological activity as the conditions vary (Bhadra, Hart, & Hall, 2014). Therefore, it is necessary to understand the unique taphonomic processes that occur when a body is concealed. While numerous types of concealment have been researched and investigated in the past, minimal research has been specifically related to concealment within a suitcase (Bhadra, Hart, & hall, 2014). This 32 literature review aims to address the effects of suitcase concealment on the decomposition rate and entomological activity of a cadaver.

6.0 FUTURE RESEARCH DIRECTIONS

Due to this being a pilot study, more replicated studies need to be undertaken and results re-evaluated in order to get a better understanding of this particular case.

Future analyses may seek to focus on pig carcasses in suitcases and other types of luggage. This literature has addressed and focuses on fabric suitcases; however, there are still many variables to investigate such as the material/type (hard case for example) and texture (nylon, polyester, and aluminium) of suitcases/luggage, the type of suitcase/luggage (briefcase, backpack, duffel bag) and whether or not different zippers are influential on the rate of decomposition/insect activity. In addition, developing alterations to this experiment with regards to season/climate, location and different environments, may aid in determining how these factors influence differentiation. When there is enough research on these factors over time, future research should seek to conduct analysis using human cadavers. This will help to ensure that any differences identified in pig carcasses can be more accurately observed in human cadavers and be applied to forensic investigations.

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- **Part Two** -

MANUSCRIPT

THE EFFECT OF SUITCASE CONCEALMENT
ON THE INSECT COLONISATION:
A PILOT STUDY IN WESTERN AUSTRALIA

THE EFFECT OF SUITCASE CONCEALMENT ON THE INSECT COLONIZATION: A PILOT STUDY IN WESTERN AUSTRALIA

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ABSTRACT

Decomposition is a complex and continuous process that involves the breakdown of soft tissues following the death event. This is often mediated by the action of macro- and micro-fauna, especially necrophagous insects belonging to the Orders Diptera and Coleoptera). Access to the cadaver is the first requirement for a decomposition process mediated by the fauna. However, in several occasions the cadaver is concealed in different manners, often to avoid an easy discovery by the authorities. The present study investigated for the first time the effects of suitcases as a barrier that may affect the decomposition of bodies in Western Australia. A total of six pig carcasses (*Sus scrofa* L., approx. 7-11 kg) were used as a substitute for human cadavers. Five pigs were singularly placed in identical fabric zip suitcases with the sixth pig acting as the control (placed on the surface of the ground inside a cage and left to decompose naturally). As expected, the study showed that the rate of decomposition was different between the control pig and concealed pigs. The rate of decomposition of the control pig followed the typical pattern- in terms of both stages of decomposition, time required for each stage and insect species involved in the process. Pigs inside the suitcases, instead, experienced a much broader range of changes, mostly characteristic of wet decomposition. However, temperature comparisons revealed no significant differences between the ambient temperature and the temperature inside the suitcases. The pattern of insect succession also varied. Carcass attendance by representatives of the Calliphoridae at the control occurred within minutes of positioning, and oviposition occurred within the first day. In contrast, Calliphoridae were not observed at the suitcases until the next day, and oviposition didn't occur until day 9. The arthropod species also varied between those found inside the suitcases and at the control, with families Phoridae and Fanniidae highly prevalent inside the suitcases. This pilot study represents the first research regarding decomposition in suitcases performed in Australia.

KEYWORDS: decomposition, forensic entomology, insect succession, suitcase concealment, PMI

INTRODUCTION

Forensic entomology is the study of insects and other arthropods that are part of a criminal case and is used as evidence (R. D. Hall, 1990). Knowledge of the insects found at a crime scene has been discovered to assist many types of forensic investigations by providing information under certain conditions on where, when, and how a crime was committed or how a person died (G. S. Anderson & Cervenka, 2002; Catts & Goff, 1992; Catts & Haskell, 1990; Greenberg & Kunich, 2002; Introna & Campobasso, 2000). Since blowflies (Diptera: Calliphoridae) are usually the first necrophagous fauna to find a cadaver, they are the most important insect evidence collected in crime scenes (Greenberg & Kunich, 2002). Different temperature and environmental conditions influence different species of blowflies and their arrival time to the cadaver.

Decomposition of a cadaver can support arthropods and large microorganisms, and generally follows a predictable sequence (G. Anderson & VanLaerhoven, 1996; Avila & Goff, 1998; Shalaby, deCarvalho, & Goff, 2000). By examining the decomposition of a body and the developmental stages of insect larvae, an estimated minimum post-mortem interval (minPMI) can be estimated, an important application in forensic entomology (Amendt, Zehner, & Krettek, 2004; Greenberg, 1991; Nuorteva, 1977; Phillips, 2012). Forensic researchers have thoroughly documented the known pattern of arthropod succession on cadavers to analyse the arthropod fauna to estimate the most accurate PMI in cases where the time of death is unknown (Amendt, Krettek, Niess, Zehner, & Braztke, 2000; Archer & Elgar, 2003; Early & Goff, 1986; Schoenly, 1992). When estimating the PMI, considering factors that may affect the rates and patterns of insect invasion on the body is important (Goff, 1992). Factors such as temperature, humidity and rainfall, and whether the cadaver is exposed or concealed have all been evident to influence the rate of insect invasion (Goff, 1992). Access to the cadaver is the first requirement for a decomposition process mediated by the fauna. However, in several occasions the cadaver is concealed in different manners, often to avoid an easy discovery by the authorities. Studies regarding the effect of different concealments are therefore needed to provide more information to law enforcement and pathologists investigating such cases.

One scenario involving the decomposition of a cadaver in a vehicle's trunk where fly access is restricted has been studied in Louisiana. The study attempted to estimate the time delay of insects finding the cadaver inside the vehicle (Catts & Goff, 1992). The study results

suggested a delay of three days; however, the results have not been published nor can be comparable to a cadaver decomposing in the vehicle's interior (Catts & Goff, 1992). A similar study has been carried on in Western Australia. Greenberg and Woolridge show that fly activity is delayed in laying egg until low light conditions are overcome (Greenberg, 1990; Woolridge, Scrase, & Wall, 2007). Studies also show that some calliphorids (*Lucilia cuprina* (Wiedemann) (Diptera: Calliphoridae)) lay eggs only when they are in contact with moisture (Bartone-Browne, 1962). Anderson, Reibe and Madea carried out studies observing the delay of calliphorid colonisation comparing bodies that were exposed indoors with those exposed outdoors (G. S. Anderson, 2011; Reibe & Madea, 2010). Results showed that colonisation was much lower on indoor cadavers. Comparing the outdoor cadavers, the cadaver in taller grasslands was slower to colonise than the cadaver in shorter grasslands (Lane, 1975). Concealment of a cadaver delays colonisation time and only certain species of blowflies can gain access to the remains (Gunn & Bird, 2011). If a cadaver is buried or wrapped, coffin flies (*Conicera tibialis* Schmitz *(*Diptera: Phoridae*))*, larvae of the Muscid genera *Muscina* and *Ophyra* can burrow through soil to reach the remains (Gunn & Bird, 2011). Wrapping a cadaver with heavy blankets can delay colonisation by 24h or more due to the blanket blocking suitable ovipositions (Ahmad et al., 2011).

The factors that affect oviposition in calliphorids help enable forensic entomologists to address concealed situations of body disposal to estimate the onset of fly colonisation. While there have been a number of studies on concealment, however, factors affecting blowfly accessibility to cadavers in suitcases has not thoroughly been studied. A study in the UK discovered that larvae can enter a suitcase through the zipper, but failed to narrow down a specific period of delay for oviposition and colonisation (Bhadra, Hart, & Hall, 2014).

To date, no research regarding concealment of cadavers in suitcases has been conducted in Australia. However, several cases of cadaver concealment in Australia are known to the news. To address this issue, this project focuses on the effect of suitcase concealment on insect colonisation in Western Australia. This study simulated the concealment of five pigs within identical suitcases compared to an exposed pig on the ground (control), specifically documenting and analysing the decomposition process, as well as entomological data from within and around the suitcases for a period of 42 days (April – May). It was hypothesised that the concealment of a corpse within a suitcase will have an effect on the rate of decomposition and has an effect on the entomological activity. The following questions will be addressed in this study:

1) Which insect species are present inside the suitcases vs. the exposed control?

2) Are there differences in success patterns of insects inside the suitcases vs. the exposed control?

3) Did the pig decompose differently when concealed in a suitcase?

Due to this being a pilot study, limits of this study may be found in the limited number of samples used as well as the period investigated. Further studies are needed, but the present may be considered the first attempt in obtaining data that could help pathologist and law enforcements investigating cases of cadaver concealed in suitcases in the Western Australian territory.

MATERIALS AND METHODS

The field of experiment was at the Murdoch University Veterinary Farm, Murdoch, Western Australia, Australia. It was conducted over a period of six weeks (April – May 2017). The site environment was eucalypt woodland on a sandy soil, with the most frequent surrounding vegetation being grass trees (*Xanthorrhoea preissii* Endl.) and zamia palms (*Macrozamia riedlei*) (Dell & Bennett, 1986).

For this particular study, a total of six pig carcasses (8.8 $\pm$ 1.2 kg) were used as a substitute for human cadavers. Pig carcasses were chosen as they are considered to best mimic the decomposition sequences of humans. The pig carcasses were obtained from Nambeelup Piggery, Mandurah and euthanized humanly in accordance with animal ethics. The carcasses were received fresh, weighed, and transported to the site.

The experimental samples consisted of pigs placed inside suitcases. A total of five identical suitcases were used over the course of the study. The suitcases were pursued in a main store, they were all identical in the model and in the colour. The suitcases were made from fabric (polyester), contained a retractable handle, zippered compartments, four wheels, and had dimensions of 72cm (H) x 47cm (W) x 24cm (D). One control sample was used in the study which consisted of a pig being placed on the surface of the ground and left outside in the open air to decompose and attract insects naturally. This pig was placed in one of the aviaries (caged structure) located at the site to protect it from large animal scavenging. The pores of the fencing were large enough for only flies and insects to travel in. Each pig was labeled with a unique number (1-6) and all six pigs were placed at least 15 metres apart from each other to prevent cross-contamination. The number of each pig also refers to the suitcase number (eg. pig 1 inside suitcase 1), with pig 6 as the control. The positioning and place for each carcass was recorded (Figure 1). The positioning of each pig was carefully planned beforehand so that they were all under similar conditions (eg. mostly shade).
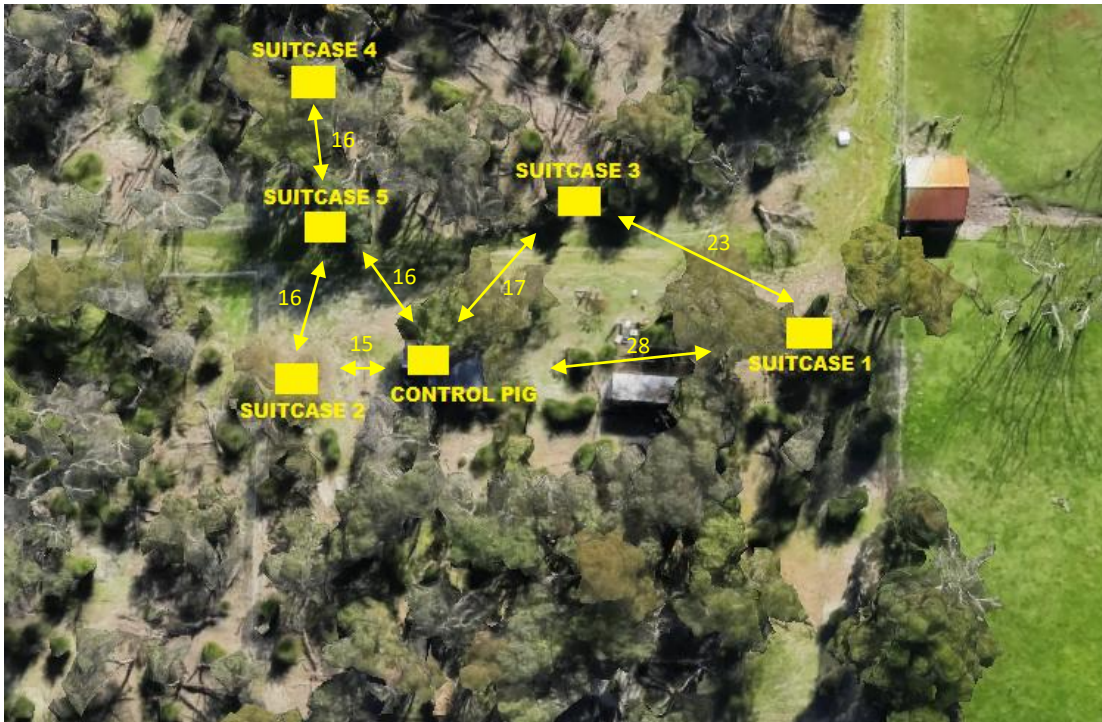
Figure 1: Location of each pig within the study site (image taken from Google Earth). All measurements in metres.

Temperature data loggers (EasyLog) were used to record the temperature and humidity within the suitcases, as well as the ambient temperature/humidity of the study field (placed near the control pig). All data loggers were programmed to record temperature and humidity every 30 minutes throughout the study period. The data was transferred to a computer for analysis at the completion of the fieldwork.

Control Pig (Exposed)

Photographs and notes were taken every day regarding the progression of decomposition until the carcass reached advanced decay stage (first three weeks). Entomological changes to the control pig were also documented each sampling day through the use of photographs, notes, and sampling of the insect material present. Eggs and larvae present were representatively sampled with a spoon or tweezers, and beetles and other crawling insects were collected with tweezers. These were placed in screw cap containers and labeled. On completion of sampling, these containers were brought back to the laboratory at Murdoch University where a subsample was killed with hot water and then preserved with 70% ethanol (Amendt et al., 2007). The majority of the samples collected were reared to the adult stage for species identification (Byrd & Castner, 2010). Eggs/larvae to be identified were reared to adult on dog food in containers covered with mesh.

From week three onwards (during advanced decay stage) to the end of the study, this process was spread out further every two to three days.

Experimental Pigs (Suitcases)

Each suitcase was opened after a defined time period. For example, suitcase 1 was opened after 14 days, suitcase 2 after 21 days, suitcase 3 after 28 days, suitcase 4 after 36 days and suitcase 5 after 42 days (mostly one week after each other).

Every sampling day (as described above) prior to the opening of each suitcase, an external examination was completed to check for any changes to the outside of the suitcase, such as adult fly interest, eggs/egg laying, larvae and/or other insect activity, and/or the leaking of decompositional fluid. Collection occurred when any eggs, larvae or any other insects/organisms were observed. Once a suitcase was opened, after photographing and written documentation had been completed, sampling occurred. If there was significant insect colonisation, the same sampling procedure that was used for the control sample was performed. If pupae were present, all different stages/colours were collected with tweezers and placed in a screw cap container. All pupae were left in the container and covered with mesh, to reach adult stage. The same also applied to decomposition with notes and photographs taken once the suitcase was opened.

Each suitcase and pig carcass was left at the site until the end of the study in which they were disposed of appropriately.

RESULTS

Temperature

Figure 1 illustrates the plot of daily mean temperatures (both ambient temperature of study field and inside suitcases) and rainfall over the 42-day period of the study. The mean ambient temperature ranged from a minimum of 13.9°C to a maximum of 21.2°C. Inside suitcase 1, the mean temperature ranged from a minimum of 17.9°C to a maximum of 21.9°C (over 14 days until opened); inside suitcase 2 from 16.2°C to 21.6°C (over 21 days) and inside suitcase 4 from 15.0°C to 21.1°C (over 36 days). As previously mentioned, the data loggers placed inside suitcase 3 and 5 were damaged during the course of the study and therefore no data could be recovered. Paired sample t-tests were performed to determine whether there were any statistical differences between the daily average temperatures recorded for ambient air and inside any of the suitcases. Results revealed no significant differences between any of the suitcases and the ambient temperature. For example, there was no significant difference in the average temperatures recorded inside suitcase 1 and the ambient temperature values ($t = -0.47$, $p = 0.005$). This was also performed between each suitcase and again revealed no significant differences. Rainfall occurred mainly towards the end of the study, including days 13 (1mm), 25 (10mm), 28 (0.5mm), 34 (3.2mm), 35 (2.8mm), 38 (10.5mm), 39 (4mm), 40 (12.5mm), 41 (6.7mm) and 42 (4.5mm). Rainfall data was obtained from the Murdoch Weather Station.
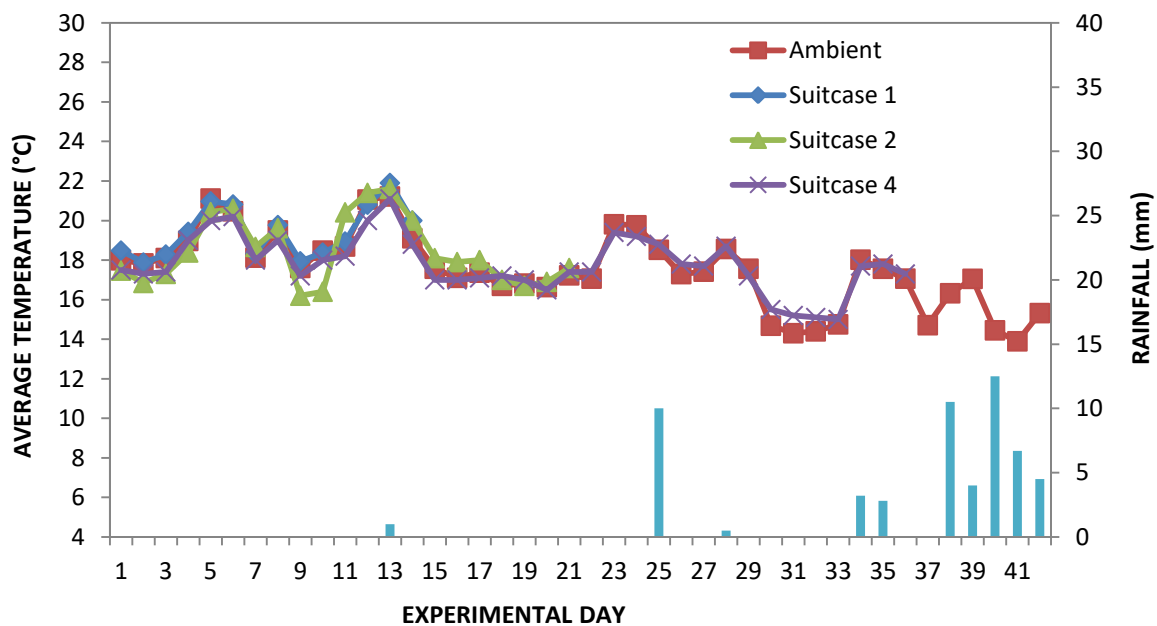
Figure 1: Climate information for the study

Decomposition

Four observable stages of decomposition were recognised for the surface-exposed control: fresh, bloat and decay (split into active and advanced decay) (Figure 2). The decomposition did not reach the fifth skeletal/remains stage during the 42 day period of the study. Figure 3 shows the duration of each stage of decomposition. The fresh stage lasted approximately two days, in which the bloat stage started. This stage lasted nine days, which was followed by active decay (duration of 10 days). For the decomposition stages, the start and end points are mostly subjective, but the start of the Decay stage is marked by a specific physical event. This is when the breaking of the skin and release of abdomen gasses occurs, due to the combined activities of the maggot feeding and bacterial putrefaction (Goff, 2009). The body deflates at this point, resulting in the beginning of the Decay Stage. Advanced decay lasted from day 21 to the end of the study.

Day 1 FRESH   Day 9 BLOAT

Day 19 ACTIVE DECAY   Day 38 ADVANCED DECAY

Figure 2: A series of photographs illustrating the stages of decomposition of the control pig

Figure 3: The duration of each stage of decomposition (in days) of surface-exposed control pig. Stages of decomposition were determined using those described in Payne (1965).

While the control followed the typical pattern of decomposition, the decomposition of pigs in the suitcases was slightly different/ unpredictable (Figure 4). Here, a much broader range of decompositional changes were experienced, most of which were characteristic of wet decomposition. The first suitcase opening appeared simil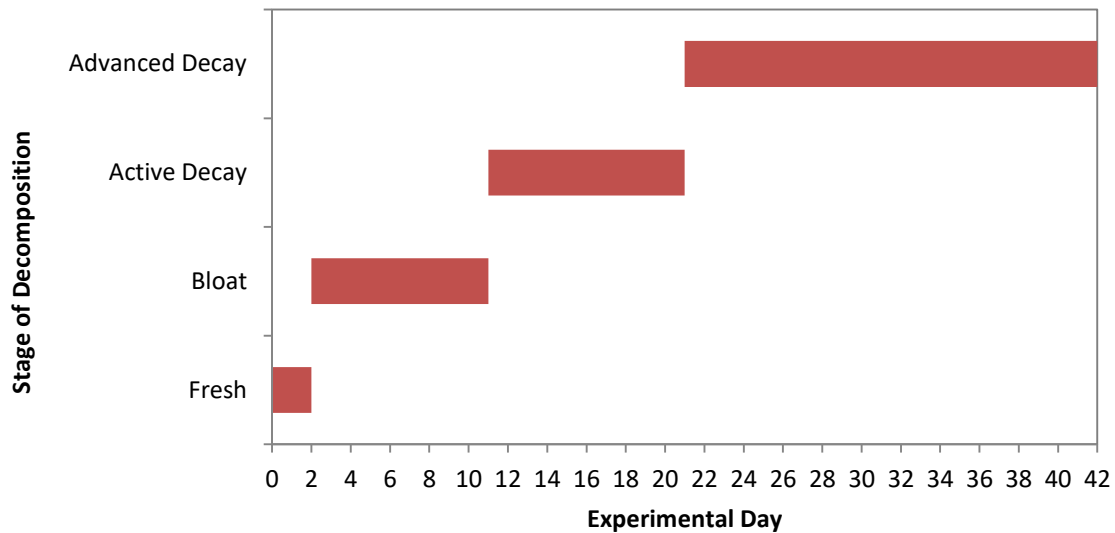ar to the control; however the progression was a little slower. The breaking of the skin was evident in the control (active decay), with the pig inside suitcase 1 still appearing in bloat phase. On day 21, both the control and suitcase 2 were in active decay. The major difference here was that the moisture content of the suitcase was a lot higher compared to the control. Up to this stage, only one millimetre of rainfall has fallen. On day 28 however, both pigs appeared very different from one another. Again this was due to the water and moisture content. The control appeared very dry with not much soft tissue left (advanced decay). In contrast, suitcase 3 contained a greater amount of water, as well as insect activity (still in active decay). The large amount of moisture inside may be due to the ten millilitres of rain received on day 25 of the study.

There was not much change for the control from day 28 right through to the end of the study (day 42), with the carcass in advanced decay. The control only appears wet in the image on day 42 due to the large amount of rain received towards the end of the study. While the control experienced a large amount of dry decomposition characterised by brown to black colour changes, suitcase 4 started to develop a toughened outer layer of soft tissue that had a brownish colouration, with the underlying tissue tender and pink in colour.

The decomposition and moisture content was less though in suitcase 4 when compared to suitcase 3. Suitcase 5 also contained skin with the same brownish colouration and appeared slightly more decomposed that suitcase 4.

Day 14 CONTROL

Day 14 SUITCASE 1 OPENING

Day 21 CONTROL

Day 21 SUITCASE 2 OPENING

Day 28 CONTROL

Day 28 SUITCASE 3 OPENING

Day 36 CONTROL

Day 36 SUITCASE 4 OPENING

Day 42 CONTROL

Day 42 SUITCASE 5 OPENING

Figure 4: A series of photographs comparing suitcases and control

Entomology

Throughout the duration of the study, a total of nine arthropod taxa were identified during the decomposition of the control (table 1), representing three orders and six families. Up to nine arthropod taxa were also identified throughout the opening of the suitcases (table 3), however, none of the five suitcases contained all nine taxa; Suitcase 1 contained six, suitcase 2 three, suitcase 3 five, suitcase 4 six and suitcase 5 five. Representatives of the order Diptera were the first colonisers of the control, closely followed by representatives of the order Coleoptera as decomposition progressed. In comparison to the suitcases, Coleoptera were observed around the same time or slightly before Diptera. In terms of Coleoptera; Histeridae, Carabidae and Staphylinidae families were present on the control, whereas only Histeridae and Silphidae were present inside the suitcases (only suitcase 4). Outside the suitcases, all the Coleoptera mentioned for control and inside suitcases were observed, as well as Forficulidae.

The insect succession pattern was basically the same between the surface control and outside the suitcases (table 2). However, differences in the timing of insect colonisation were clearly evident. Adult flies were attracted to the control pig within minutes of positioning, while no fly activity was observed around any of the suitcases until approximately the next day (day 1). Observations and collection of eggs indicated a distinct lag in the timing of oviposition between the control and outside suitcases. Eggs of the blowfly species, *Calliphora albifrontalis* Malloch (Diptera: Calliphoridae) and *Lucilia sp.,* were observed inside the mouth and behind the left ear of the control on day 1. In comparison, eggs were not observed outside any of the suitcases until day 9. Larvae were first present on the control pig 5 days after initial exposure, however, larvae were not present outside the suitcases until day 9.

A major difference between the control and inside suitcases was that Phoridae were present inside most of the suitcases; however, they were not present at all on the control pig. Another notable difference was that Fanniidae were present inside all suitcases but were only observed on the control on day 17. Both these families appeared to be the first colonisers inside suitcases as both larvae and pupae were observed and collected inside the first suitcase opened.

DISCUSSION

Changes in climatic factors such as temperature, rainfall, and relative humidity affect the rate of carrion decomposition which alters the number of days to complete decomposition (De Carvalho & Linhares, 2001). This study was performed over the autumn season (April – May) in the suburban area of Perth, Western Australia. The exposed pig completely decomposed in 42 days; however, the study was not long enough for the pigs in concealed suitcases to fully decompose. In Western Australia and other regions of the world, the number of days recorded in cases for exposed pigs to decompose were as the following; 40 days in Western Australia (Bornemissza, 1957), 14 days in Malaysia (Chin, Marwi, Salleh, Jeffery, & Omar, 2007), 25 days in Hawaii (Payne, 1965), 40 days in Brazil ((De Carvalho & Linhares, 2001), and 83 days in Columbia (Martinez, Duque, & Wolff, 2007). The above differences are usually a result from unique geographic climatic conditions (Sharanowski, Walker, & Anderson, 2008). Previous research has identified differences in insect succession patterns between different seasons and geographic regions in Western Australia for surface carcasses (Voss, Spafford, & Dadour, 2009). The effect of temperature on the developmental rate of insects is widely known (Ames & Turner, 2003; Greenberg, 1991). Insect activity and larval development rate will increase as temperature increases, and this ultimately increases the rate of decomposition (Shean, Messinger, & Papworth, 1993). However, in this study, the results in Figure 2 did not show a significant difference between the temperatures of the surface exposed pig (ambient temperature) and the concealed suitcase pigs. This could be due to the fact that this study was designed for all the pigs to be under similar conditions (all pigs were placed in shaded areas). The data loggers used were not effective in obtaining temperature for suitcases 3 and 5, possibly due to rainfall and flooding of the suitcases, resulting in loss of data. However, following the observation of the obtained data, it is possible to infer that a similar temperature was experienced inside suitcases 3 and 5. Colonisation of the pig was delayed evidently on days that had rainfall, resulting in a low succession activity rate and not many visible flies/eggs surrounding the sites, having a negative influence on the decomposition rate.

The second most important variable affecting the decomposition rate of a cadaver, after temperature, is the access of the insects to carrion (Mann, Bass, & Meadows, 1990). If access is given, within the first few hours the female adult insects will oviposit on the carrion (D. Hall, 1948). In this study only one pig was exposed in a cage outdoors (control pig) and the remaining five were concealed in similar suitcases. All natural orifices (ears, eyes, mouth,

nose and anal regions) were fully accessible to insects. Odours from the pigs attracted adult blowflies within minutes of positioning the pigs, leading to an early oviposition in the natural orifices. On day 1, eggs (*C. albifrontalis* and *Lucilia* sp.) were found in the control pig's mouth, however, no eggs were found on any of the suitcases. At the end of the first week there were masses of larvae feeding on the control pig.

Goff compared studies of a carrion outdoor verse a carrion indoor, and there was significant differences in insect colonisation (Goff, 1991). It was evident that certain flies would be discovered only on the outdoor carrion. This research can be used for cases of body transportation from indoors to outdoors. In table 3, *C. albifrontalis* was only found in the first opened suitcase (suitcase 1).In table 2, *Fanniidae ss.pp.* and *Calliphora dubia (*Macquart*)* were exclusively found on the exposed control pig. In addition, insects belonging to the family Phoridae were exclusively found in suitcase 3.

In many forensic investigations blowflies are usually the primary colonisers on a body. In Western Australia, blowflies (Diptera: Calliphoridae), such as, *Lucilia sericata (*Meigen*)*, C. *dubia*, *C. albifrontalis*, and *Chrysomya megacephala* (Fabricius) have all been identified as primary colonisers. While depending on the season, *Chrysomya rufifacies* (Maquart) and *Chrysomya varipes* (Maquart) are usually considered secondary colonisers (Voss, et al., 2009). During winter in Egypt, *Calliphora vicina (*Robineau-Desvoidy*)* was the most abundant, *L.sericata* was dominant during the fall, winter and spring seasons and *Chrysomya albiceps* (Wiedemann) in summer, spring and fall (Tantawi, El-Kady, Greenberg, & El-Ghaffar, 1996). In the southern United States, *Phaenicia cuprina* (synonymous for *L.cuprina*) was abundant in summer (Goddard, 1988; D. Hall, 1948). Understanding the seasonal differences in insect succession can be used for corpses that are found years after death, since pupae cases and beetle skin can be dated to estimate the season of death (Strong & Adams, 1990).

Three arthropod orders, six families and nine species were collected and identified over the duration of this study. Of these, four families from Diptera (Phoridae, Fanniidae, Muscidae, Calliphoridae) used the sufficient 10.4kg pig carrion for feeding and breeding purposes. From the table 1 *C. albifrontalis* and *C. Lucilia* sp. were the first colonisers on the control pig. Research has shown a wide range in numbers of arthropods collected from different geographic regions and types of carrions. For example, in British Columbia, *Lucilia illustris* Meigen was the first species to arrive on a pig carrion within minutes of exposure, in contrast

to a study in Manitoba, *Phormia regina* Meigen was the first (G. Anderson & VanLaerhoven, 1996). *Calliphora vomitoria (*L.*)* were the first species to colonise a rabbit carrion after three days of exposure in northern France (Bourel et al., 1999). In Egypt, *C. vicina* and *L. sericata* were the first coloniser in the study of Tantawi et al. (Tantawi, et al., 1996).

A body concealed in a suitcase has fewer egg deposition sites for the female flies. During observations all the suitcases had eggs deposited around the lining of the zippers and sometimes underneath the suitcases on the ninth day, in comparison with the exposed pig having egg deposits on day 1. Ants were evidently removing eggs from the suitcase; this could have delayed the observation of eggs deposited on the suitcase. Parasitic wasps (Hymenoptera: Vespidae), injects their egg inside the fly pupae (metamorphosis stage) and once the wasp egg has hatched it then feeds on the pupae before it ejects out into the world. These wasps were present on the control pig most days; however, they were only present in two suitcase opening (suitcase 1 - day 14, and suitcase 5 - day 42). Coffin flies (Diptera Phoridae) latrine flies (Diptera Fanniidae), as well as common house flies (Diptera: Muscidae) and blowflies (Diptera: Calliphoridae) were all present in most of the suitcases. Fanniidae and Muscidae were the only two that infested all of the suitcases. Coffin flies, are an important species in relation to buried bodies, as they are known to burrow down to reach the buried body (Benecke, Josephi, & Zweihoff, 2004). It is evident that these species are small enough to get inside a concealed suitcase through the zippers and the gaps between the attached wheels on the bottom of the suitcase. Larvae and pupae were found outside the suitcase, particularly underneath. This could be from escaping the suitcase through the gaps mentioned above.

This study has focused on only one similar suitcase and the effect it has on insect succession. The zip teeth are one of many factors that affect the ability of colonisation. It would be beneficial to examine the differences in different constructed suitcases (zippers and the inner lining), this will help with post-feeding and larvae escaping. Since there are differences in each region, estimations of time of death can be inconclusive if compared worldwide. Therefore, it would also be beneficial to have multiple studies in different seasons and in different regions to obtain a more accurate time of death, location of death, and if the body has been moved (R. D. Hall, 1990).

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Table 1: Time of arrival and duration of stay of the arthropod collected from the control pig and outside the suitcases

Table 2: Arthropod collected (including the different stages) from the control pig over the course of the study

Order	Family	Genus	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	21	23	25	28	30	33	36	38	42
Hymenoptera	Vespidae	ss.pp.									X	X	X				X	X	X	X	X	X	X	X	X	X					X	
Coleoptera	Carabidae	ss.pp.									A	A	A	A	A																
Coleoptera	Staphylinidae	ss.pp.																					A	A	A	A	A	A	A	A	A
Coleoptera	Histeridae	ss.pp.										A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Diptera	Faniidae	ss.pp.																		L											
Diptera	Muscidae	ss.pp.																L	L	L		L	L	L	L	L	L	L	L	L	L
Diptera	Calliphoridae	*Chrysomya*	sp.								L	L	L		L	L	L	L	L	L	L	L	L	L	L	L	L,P	P	P	P	P
Diptera	Calliphoridae	*Calliphora*	*dubia*					L	L	L		L	L																		
Diptera	Calliphoridae	*Calliphora*	*albifrontalis*	E	E	E	E	E, L	L	L		L	L	L			L		L	L	L										
Diptera	Calliphoridae	*Lucilia*	sp.	E	E	E	E	E, L		L	L	L	L																		
	Days of collection			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	21	23	25	28	30	33	36	38	42

Legenda	E = Eggs; L = Larva; P = Pupa; A = adult; X = presence.

Table 3: Arthropod (including different stages) collected from inside the suitcases

Order	Family	Genus	Species					
Hymenoptera	Vespidae	ss.pp.		X				A
Coleoptera	Silphidae	ss.pp.					L	
	Histeridae	ss.pp.					A	
Diptera	Phoridae	ss.pp.		L, P		P	P	
	Faniidae	ss.pp.		L, P	P	P	P	P
	Muscidae	ss.pp.		E, L	L	L	E, L	L
	Calliphoridae	*Chrysomya*	sp.		L, P	L, P*	L, P	L, P
		Calliphora	*albifrontalis*	L*				
		Calliphora	sp.	L		P		L, P
Day of suitcase opening				14	21	28	36	42
Legenda:	E = Eggs; L = Larva; P = Pupa; A = adult; X = presence; * = parasited.							