Decomposition and Insect Succession on Hanging Carcasses in Western Australia

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Declaration

I declare that the research presented in this thesis, for the Master of Forensic Science at the University of Western Australia, is my own work. The results of the work have not been submitted for assessment, in full or part, within any other tertiary institute, except where due acknowledgement has been made in the text.

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Abstract

The accuracy of entomological based determination of minimum post-mortem interval (mPMI) is greatly improved where the particulars of the death scenario are closely represented by the available reference data used. This study aimed to provide reference data on decomposition and insect succession for hanging carcasses in Western Australia. Decomposition processes and insect colonisation of hanging pig carcasses were compared with carcasses decomposed on the soil surface. As seasonal conditions, particularly temperature, are known to influence both the rate of decomposition and insect succession, this work considered the effect of temperature and season by conducting trials over three different time periods of the year during autumn and spring.

The decomposition of carcasses while hanging was markedly different to decomposition of carcasses lying on the soil surface and notably affected insect succession patterns both across trials and, to a lesser extent, within trials. Decomposition of hanging carcasses differed when compared with surface carcasses in respect to overall retention of carcass biomass throughout the stages of decomposition. This observation was consistent throughout each of the trials, although the observed retention of flesh increased for all treatment groups during the cooler trial periods. The comparatively prolonged retention of flesh and rapid mummification of hanged carcasses is likely attributed to altered climatic conditions following physical suspension above the ground and the reduced larval massing observed on hanging carcasses. Hanging carcasses supported much smaller larval masses compared to surface carcasses and the corresponding period of larval activity on hanging carcasses was shorter. Differences in seasonal assemblages of the insect fauna were apparent between trials and were consistent with expected seasonality of species known to be present in the region.

Within trials the colonising taxa were consistent between hanged and surface carcasses. Differences were, however, evident in species arrival time and duration between hanged and surface carcasses. Hanging carcasses appear to

support lower numbers of *Ch. rufifacies* and *Ch. varipes* which corresponded with a prolonged colonisation of smooth maggots on hanging carcasses compared to surface carcasses. Where the predatory species, *Ch. rufifacies* and *Ch. varipes*, dominated surface carcasses other dipteran taxa were largely displaced. In contrast, on hanging carcasses, initial colonising smooth maggots (*C. albifrontalis, C. dubia, L. sericata and C.megacephala*) were observed to persist and thrive for a longer period of time compared to the same species on surface carcasses.

Finally, the drip zone, an area beneath a suspended carcass where fallen fluid, tissue and insects accumulate, has previously been reported as a reliable source of insect evidence for use in the estimation of mPMI. Here we report high variability in the initial formation timing of the drip zone and uncertainly in origin of colonising insects (oviposition may occur directly onto the tissue in the drip zone or occur on the hanged carcass and fall into the drip zone). As such, the use of insect evidence collected from the drip zone is not supported by this study as a reliable indicator of mPMI and considerable error could be introduced to the calculated mPMI where such estimates are generated for hanging cases. The data detailed here provides a reference point for forensic case work in Australia involving hanged remains and identifies the importance of matching the specifics of the death scene to the closest available reference data.

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Introduction

1.1 Project Aims

Forensic entomology is the branch of science relating to the use of insects in legal investigations. Forensic entomology is primarily associated with estimations of time since death, also known as minimum post mortem interval (mPMI) in cases of homicide, suicide, or unexplained death (Voss et at., 2009; Eberhardt & Elliot, 2008). Knowledge of relevant insect succession patterns has proven invaluable to investigators as a tool in helping establish mPMI and/or post mortem movement of a body in many cases where relevant reference data is available (LeBlanc & Strongman, 2002; Amendt et at., 2010; Higley & Haskell, 2010; Vanlaerhoven, 1997). Problematically, due to the wide variation of death scenarios and locations, there is clear disparity between current available succession data and its applicability to forensic case work. Application of data that does not match the death scenario or geographic region of the crime can lead to erroneous mPMI estimates. For this reason, there is a continued need for entomological research relevant to death scenarios to assist with making correct mPMI estimates.

In Australia, entomological studies of insect succession patterns onto decomposing remains have been published for various death scenarios, with the majority of work investigating decomposition of carcasses placed directly on the soil surface (Wallman & Archer, 2002; Archer & Turner, 2003; Voss et al., 2008; Voss et al., 2009; Voss et al., 2011; Johnson & Wallman, 2014). Such works have highlighted the importance of generating data which reflects annual and seasonal variation in patterns of insect succession (Archer, 2004a; Voss et al., 2009). The effect of temperature on growth and developmental rate of insects is widely known (Greenberg, 1991; Ames & Turner, 2003). High temperatures and humidity will increase insect activity and larval development, and this will increase the rate of carcass decay (Shean et at., 1993). Whilst cooler temperatures will inhibit larval growth, and delay the onset of decay (Matuszeweski et at., 2010a). A majority of forensic entomology reference data is collected from limited time periods, usually a

single season, even though the importance of work conducted over different times of the year is stressed within many of the forensic entomology manuals (Goff, 2010; Byrd & Castner, 2010; Gennard, 2012).

One death scenario that currently lacks sufficient reference data for use in mPMI estimation is the decomposition of a body while hanging. Hanging remains the primary cause of death through suicide and accidental deaths (Chin et at., 2010; Australian Bureau of Statistics, 2015). During 2012, hanging accounted for 54.4% of all suicide deaths followed by poisoning by drugs which accounting for a mere 14.5% of all cases (Australian Bureau of Statistics, 2015). In 2012, over a quarter of the deaths in males aged 20-24, 25-29 and 30-34 were due to suicide (28.7%, 26.5% and 27.5%, respectively). For females during 2012, the age group is slightly lower with woman between 15-19 years and 20-24 years, suicide accounted for 32.6% and 25.5% of deaths in these age groups respectively (Australian Bureau of Statistics, 2015).

This project aims to compare decomposition processes and insect succession patterns across seasons for hanging and surface laying pig carcasses in Western Australia. Data will compliment and expand on the limited existing reference data for a hanging death scenario and document the process for the first time in the Australasian region. The resulting data will provide greater accuracy in the entomological estimation of mPMI where investigations involve death by hanging in Perth, Western Australia (WA).

1.2 **Aims**

The main objective of this study is to document the decomposition process and provide reference data detailing insect succession onto hanging carcasses in Perth, Western Australia. The specific objectives are as follows:

I. **Document decomposition processes of hanging and surface laying (control) carcasses in Western Australia (WA).**

II. **Compare insect colonisation and succession patterns between hanging and surface lying carcasses in WA**

III. **Compare insect succession and decomposition processes undervarying weather conditions in WA (early autumn, late autumn, and spring)**

IV. **Establish reference data for use in forensic case work pertaining to hanging carcasses in WA**

Literature Review

2.1 Forensic Entomology

The primary basis of mPMI estimation involves understanding the biology, behaviour and colonisation patterns of insects found in association with decomposing carcasses for a given set of geographical and environmental conditions (Morris & Dadour, 2005). It is commonly accepted that insect succession (the arrival and departure of insects) onto a decomposing carcass happens in a predictable manner, and this predictability can be used to assist in the estimation of mPMI (Voss et at., 2009).

Forensic entomology is applicable in many different situations including veterinary and wildlife forensics (Anderson, 1999), illegal trafficking of cannabis (Crossby et al., 1986), housing disputes (Goff, 2000), or cases of neglect (Beneke, 2003). However, the most widely known application is in relation to medical entomology and the use of insect evidence to assist in cases of homicide, suicide, or unexplained death. Where insect material is collected from human remains as part of a forensic investigation an entomologist can provide investigators with a time since death estimate to assist in the reconstruction of events surrounding the death and to focus further investigation (Anderson, 1995; Amendt et al., 2010).

Whilst modern day advances in forensic entomology are relatively novel within the scientific community, the basic concept of using insects in criminal investigations is not new to science. The first documented case of forensic entomology comes from the 13th century. The Chinese lawyer and death investigator Sung Tźu, reported the case in the manual titled “The Washing Away of Wrongs”. In a translation by McKnight in 1981, Tzu described investigators trying to solve the case of a murdered farmer. Investigators asked farmers in the region to line up their tools and lay them on the ground. Blow flies were attracted to one of the tools and it was assumed this was due to minute traces of the victim’s blood left on the tool. Confronted with this information, the owner confessed to the crime (McKnight, 1981)

The use of modern forensic entomology techniques became widely accepted after the successful use of insect evidence during a famous case in Britain in 1935 (Snyder Sachs, 2001). Dubbed “The Devil’s Beef Tub’ after police recovered nearly

70 pieces of human tissue from a Scottish ravine, the forensic pathologist faced the hard task of understanding what happened during the course of the murder. Critical evidence recovered from forensic entomologist Robineau-Desvoidy, enabled investigators to establish blowfly eggs were laid on the body parts prior to being dumped in the river. This information, combined with other forensic evidence recovered from the scene lead to the arrest and conviction of the perpetrator, making this case one of the first to use modern forensic entomology concepts as a legal tool in convicting someone of a crime (Morris & Dadour, 2005). The outcome of this case propelled the foundations of forensic entomology research and highlighted the need for further work in the field to increase understanding in a legal context.

In the past 30 years a number of important text books on forensic entomology have been published (Beneke, 2001). The British Museum published the first in 1986 as a procedural guide in forensic entomology directed at entomologists (Smith, 1986). Such publications have helped to advance the acceptance of forensic entomology in criminal cases and improved the relationship between lawyers and practitioners of forensic entomology in sharing common ground when discussing methodologies of criminal cases involving entomology evidence in court (Morris & Dadour, 2005). The level of acceptance of forensic entomology by the courts depends on results of scientific research, and reliability of data. As such, many recent efforts have focused on refining previous work done on larval development rates of forensically important flies (Beck, 1983; Higley & Haskell, 2010, Anderson, 2000). Other areas of research have focused on determining the duration of life stages of locally relevant species of fly at set temperatures so larval development rates can be calculated correctly when needed (Grassberger & Reiter, 2001; Grassberger & Reiter, 2002; Cook & Dadour, 2011). Molecular techniques have also advanced for the correct identifications of fly species collected off carcasses (Oliveira et al., 2011; Farncombe et al., 2014). Whilst maggots have been used to detect drugs used prior to death (George et al., 2009)

Currently, several different areas of forensic entomology are recognised based on the type of legal situation in which the science is applied. Typically there are four common scenarios to which forensic entomology becomes applicable: wildlife cases, urban dwellings, stored-product disputes and medico-legal cases (Lord & Stevenson, 1998; Catts & Goff, 1992; Morris & Dadour, 2005). This review will primarily focus on

the medico-legal use of entomology, which involves the use on insects in solving criminal, medico-legal cases such as, assault, neglect, or murder.

One of the most important factors of medico-legal forensic entomology is the establishment of time since death (or in cases of neglect or assault – the time since the abuse began) (Beneke, 2001). Where insect evidence can be used to help establish a mPMI, the information can be crucial to a criminal investigation as it may provide a focus of direction for investigators whilst assisting in the inclusion and exclusion of individuals (Wells & LaMotte, 2010). Estimates of mPMI using insects is based on knowledge of the biology and behaviour of relevant species frequently found in association with human remains including the expected arrival time of species colonising the body and the age of the colonising insects collected. It is widely agreed upon that the combined processes of decomposition and insect colonisation, termed succession, are closely linked (Fuller, 1934; Voss et al., 2010, Voss et al., 2011, Cockle & Bell, 2015). Understanding the process of decomposition, and how this can potentially influence insect attraction to and arrival at a body, is critical to the application of forensic entomology techniques.

2.2 **Decomposition**

The “Goff Model” of decomposition describes decomposition as a continuous process which begins at the moment of death and finishes when the body is completely reduced to a skeleton (Goff, 2010). Although the process is a continuum, recognisable stages of decomposition have been described in several forensic entomology manuals to delineate key events in the process (Greenberg & Kunich, 2005; Gennard, 2012).

The majority of the work detailing the process of decomposition has come from carcasses laying laterally on the soil surface (Mann et al., 1990; Beneke, 2001; Campobasso 2001; Goff, 2010, Gennard, 2012), and has been described for decomposition under temperate to warm climatic conditions using pig carcasses as a model for human decomposition (Catts & Goff, 1992). As a corpse decomposes it goes through a series of physical, chemical and biological changes (Byrd & Castner, 2010). Five stages of decomposition are generally accepted, these are known as; fresh, bloat, decay, dry and the skeletal remains stage (Amendt et at., 2010; Goff, 2010). Insects are known to be attracted to a body within minutes

after death (Archer, 2014; Anderson & VanLaerhoven, 1996), unless conditions at the crime scene (e.g., burial, concealment) delay insect colonization. Decomposition stages and the succession of insects onto and off the carcass are strongly linked (Simmons et at., 2010; Michaud et al., 2012). As a body decays, it provides a temporary, rich source of food and shelter for insect species. As well as the chemical and physical changes occurring with decomposition, colonizing insect species gradually change the state of the carcass so that different species are attracted to, and colonise the body at different stages of the decomposition process (Voss et al., 2011).

Fresh decomposition starts from the point of death, and the end of this stage is marked by the first sign of bloating (Goff, 2010; Voss et al., 2011; Gennard, 2012). The changes that take place immediately following death are known as early post-mortem changes, and these are usually more rapid than those occurring in late stage decomposition. It is during the fresh stage of decomposition that a majority of the processes occur internally. At this point primary insect colonizers which are attracted to the body will begin to oviposit in the natural orifices (Goff, 2010). Wounds inflicted prior to death also become primary sites for oviposition (Cross & Simmons, 2010). As skin splitting and other signs of advanced stages of decay are not present during the fresh stage of decomposition (giving maggots secondary entry points to the body), maggot activity during this stage is usually internalised in the natural orifices, with little activity occurring on the surface of the carcass (Goff, 2010).

The onset of the bloat stage of decomposition is characterised by putrefaction (Goff 2010; Gennard, 2012). Anaerobic bacteria in the gut and other parts of the body begin to digest the tissues producing gasses which begin to inflate the carcass. As the bloat stage reaches its peak the body is known to be in ‘full bloat’ and the body will often assume a balloon like appearance. Flies (Diptera) from the taxonomic family Calliphoridae are extensively associated with carcasses during this stage of decomposition (Goff, 2010). Different species of flies will arrive on the carcass to oviposit, and primary species will begin to form maggot masses which compete for the food source. The metabolic activity of the maggots during this time can cause the internal body temperature to increase significantly (Goff, 2010). Furthermore, as pressure builds internally, fluids seep from the natural body openings and into the

substrate below, resulting in changes to the naturally occurring soil fauna (microbes) & flora in contact with the carcass as the pH is affected from the seeping fluids (Forbes & Dadour, 2010; Goff, 2010).

The start and end points of the decay stage of decomposition are often not clearly defined by events occurring during the process of decomposition (unlike the bloat stage for example). Kreitlow (2010) wrote that the decay stage of decomposition begins when the body deflates whilst Goff (2010), states it begins when the carcass abdominal area splits and internal gasses are released - deflating the body. Gennard (2012), however, suggests decay begins when the skin splits and starts to slough off. At any rate, researchers are in agreement that this stage of decomposition begins when anaerobic bacteria in the gut of the animal has reached a peak of activity and other physical processes take over, such as maggot massing, and removal of skin from the carcass due to maggot feeding. All research reports that the decay stage of decomposition is the period of the most insect activity, and associated with strong odours of decay (Byrd & Castner, 2010; Goff, 2010; Gennard, 2012). Beetle species that began arriving during the bloat stage often become predominant during the decay stage of decomposition, and will readily be seen predating on the maggot masses (Matuszewski & Szafalowicz, 2013). In addition to the predators, necrophagous species of beetle will also arrive, and by the end of the decay stage of decomposition, the assemblage of insect species present on the carcass will have removed a majority of the flesh and fatty tissues from the body, leaving only skin and cartilage (Goff et al., 1988; Goff, 2010; Gennard,2012).

The post decay stage of decomposition is characterised by a state of reduced skin & cartilage remaining on the carcass. At this point there may be visible bones with most of the moisture removed from the body. Dipteran species normally cease to be the predominant fauna of the carcass, and various species of beetle reportedly begin to dominate the carcass (Matuszewski & Szafalowicz, 2013). At this point, the remaining skin and cartilage are removed from the body as a result of the feeding activity of beetles.

The skeletal stage is entered when only bones and hair remain at the scene. This can sometimes take years to occur and typically no obvious carrion-feeding taxa can be seen at this stage. Various soil dwelling species have been shown to play a role

in determining mPMI at this point, but as the time passes, soil pH will return to normal, and a gradual return to the original soil composite will begin. There is no definite end point for the skeletal stage of decomposition (Goff, 2010).

It is widely known that there is a link between the rate and progression of decomposition and insect succession (Rodriguez & Bass 1982; Cockle & Bell, 2015). If insects have access to a decomposing carcass, their activity will have an effect on the process of decomposition. Kočárek (2003) identified fly larvae as the primary contributors in biomass loss off a carcass during the initial stages of decomposition. Beetles and their larvae were identified as the major contributors of biomass loss in the latter stages of decomposition. Kočárek (2003) concluded that when a carcass is exposed to insects, decomposition tends to be more rapid compared to when a body remains isolated from insect activity (for example: sealed container or fridge).

A component of mPMI estimation is based on the insect community expected to occur in relation to the stage of decomposition and the predictability of insect succession onto a carcass. A number of studies have documented the relationship between insect activity and decomposition (Reed, 1958; Nuorteva, 1977; Rodrigues & Bass, 1982; Reibe & Madea, 2010; Voss et at., 2011). Before estimating mPMI it is important to understand how the process of insect succession works, and its relationship to decomposition. Several factors also influence the species community and colonisation processes of a carcass, therefore it is important that these factors are also incorporated when making mPMI estimates using insect evidence as mistakes can be made otherwise (Knight, 1995).

2.3 **Insect Succession**

Several species of insects play an essential role in the process of decomposition (Bornemissza, 1956; Megyesi et at., 2005). It's these species which have evolved to rely of the natural occurrence of death and use the available resources of a decomposing carcass as a temporary habitat and food resource (Voss et at., 2010). Insects, especially flies are usually the first to arrive at the scene of a decomposing carcass. Female flies will naturally seek out moist hidden places like the natural orifices of a recently deceased animal or person to lay their eggs/larvae for development in the required environment (Kreitlow, 2010; Goff, 2010). The timing

and order in which species arrive at a body and eventually depart is known as insect succession. It is thought that this process occurs in a predictable, orderly sequence which is closely linked with the order of decomposition (Easton & Smith, 1970; Anderson & Vanlaerhoven, 1996; Anderson, 2010a). This predictable process of insect succession can be used by investigators to assist in estimating mPMI in cases of suspicious death, murders or produce time lines for coroners reports into cases of neglect in humans and animals.

Different species of carrion insects have developed highly specific life cycles and biology, likely due to high levels of interspecies competition (Mcleod & Anderson 1992; Fisher et al., 1998). These differences include variation in seasonal life-cycle development times, habitat preference, carcass size requirements, host species and preferred stage of decomposition for colonisation (Goff, 2010). The highly specific, adapted nature of carrion species means that different species will be attracted to the decomposing body at different intervals (Nuorteva, 1977; McLeod & Anderson, 1992). Currently, it is not clearly known what attracts insects to a decomposing body, although it is widely accepted that female carrion flies are attracted to the volatile chemical cues emitted during the decomposition process (Ignell & Hansson, 2005). As chemical molecules emitted during the process of decomposition change as the body decays, it is thought these changes attract different species of insect to the carcass as it progresses through decomposition. However, the arrival of species will also effect the rate of decomposition and change and alter the carcass making it more suitable to different species as the process continues. It is this process which acts as the foundation for insect succession. Specific insect species are labelled as primary, secondary or tertiary colonizers, depending on the sequence of arrival time and oviposition (Lane, 1975).

The insect community associated with a decomposing carcass primarily consists of the flies (Diptera) and beetles, with representatives of the Diptera, notably blowflies (Family: Calliphoridae), being the most commonly found carrion insects (Mann et at., 1990). As blowflies and other species of insects colonize a carcass, their interactions with the body have an impact on its physical state, changing the environment allowing other competing insects to colonize in the process (Greenberg, 1991). Primary colonizer fly species are generally all capable of causing myiasis in humans or animals (Morris & Dadour, 2005). They are the first species to arrive to the

carcass and lay eggs or larvae during the fresh stage of decomposition. Their development and eating habits begin to alter the rate of decomposition of the carcass and this intern makes the environment more suitable for secondary coloniser species. Maggots on the carcass at this time will be in direct competition of one – another for the food resource as secondary coloniser species are known to predate primary colonisers (Kreitlow, 2010).

Insect succession is also influenced by several abiotic and biotic factors (see section 2.5), although the typical insect succession process follows a prescribed format which encompasses four simple relationships between the insects and the carcass. This includes the primary colonisers; parasitic and predatory species; omnivorous species; and adventive species of insects. Primary colonisers are typically the necrophagous species which feed directly on the carcass. This group includes many of the true flies, particularly the blow flies, and some flesh flies (Family: Sarcophagidae) (Nuorteva, 1977; Hall & Doisy, 1993). This group is considered the most important for use in estimating a minimum period of insect activity on the body during the early stages of decomposition (Goff, et al., 1998). Once primary colonisers are established on a carcass, parasitic (insects which use other insect as a host) and predatory species become the second most important group as they are directly related to the primary colonisers. Their presence on the body directly relates to the primary coloniser species as they are either adapted to parasitise them, or eat them. Occasionally this group will become important in establishing time of death estimates in late stage decomposition. This group includes many of the beetle species (Coleoptera: Silphidae, Staphylinidae, and Histeridae). The third group of insects common in a succession time line are known as omnivorous species. These species of insects feed on the corpse and the associated arthropods. Omnivorous species are known to significantly alter the rate of flesh removal from a decomposing carcass by depleting the number of necrophagous species associated with the body (Early & Goff, 1986). This group includes wasps, and ants, and some beetles. The fourth relationship includes adventive or accidental species. These are the insects which are found on or near a carcass only because the carcass is located in a position to form a natural extension of these insects normal habitat. This may include species representing the Lepidoptera, and Collembolla taxa (Goff, 2010).

Blow flies are usually the first insect species to visit a body and in many forensic investigations are known as primary colonisers. In Western Australia the blow flies (Diptera: Calliphoridae), *Lucilia sericata* Meigen 1826*, Calliphora dubia* Macquart 1855*, Calliphora albifrontalis* Malloch 1932 and, *Chrysomya megacephala* Fabricus 1794 have all been previously identified as primary colonisers. While seasonally dependent, *Chrysomya rufifacies* Macquart 1842 and *Chrysomya varipes* Macquart 1841 are usually considered secondary colonisers (Voss et at., 2009).

There are two components involved in determining mPMI based on insect evidence. The age of immature insects collected from discovered remains is determined and combined with the expected arrival time of the colonising female insect that oviposited the collected specimens. This approach requires modelling the relevant time frame based on an understanding of the predictable developmental rates of insect species for a given temperature and the adults expected arrival time at remains under for the circumstances surrounding that specific case (Anderson, 2001; Goff, 2010). As such, the accuracy of predictive modelling requires that the specific biology and behaviour of the relevant species be known under various abiotic and biotic conditions to allow accurate predictions and thus reliable mPMI estimates.

Understanding the development and lifecycle of forensically important flies, is the initial foundation for understanding mPMI calculations in forensic entomology. Flies complete a number of stages in their lifecycle; the egg stage, larval stage; pupal stage; then mature adult stage. This processes of development from egg to mature adult is known as metamorphosis. Flies undergo the most advanced form of metamorphosis known as holometabolous (Amendt et al., 2010). Holometabolous metamorphasis is characteristic of four major changes in anatomy throughout the lifecycle of the insect. Beetles, moths and butterflies all share similar complex, holometabolous lifecycles. In flies, the egg develops into a maggot, which then goes through three larval stages known as instars (1st, 2nd and 3rd instar). During the third instar phase, the maggot will migrate away from the food source (if conditions allow) to pupate (Anderson 2005a). Maggots will pupate on the body, if conditions do not allow for migration away from the carcass. Anderson (2005a) showed that migrating larvae of several Calliphoridae species were unable to climb the smooth walls of a bathtub when trying to leave a carcass that had been placed in the tub. The larvae

eventually pupated on the body, nearly covering the remains with pupae and puparia. Some species of flies will prefer to pupate closer to the carcass or food source and this is common of *Chrysomya* species or the 'hairy maggot' whilst some *caliphorid* or 'smooth maggots' species are known to wander distances up to 6 meters from a carcass (Archer & Elgar, 2003). During pupation, the pupae case will change from a light reddish brown to a darker brown, nearly black colour. The larvae will complete its transformation into an adult fly within the pupal casing and emerge once fully grown. The time it takes for this transformation to take place is individual for each species of fly. When the adult is ready to emerge, they will push off the operculum or 'cap' of the puparium using a blood-inflated area on its head called a ptilinum. This "airbag" like structure protrudes from the anterior dorsal region of the head and later shrinks back into the facial structure leaving behind a crease known as the ptilinal suture just above the antennae. Once the fly is free of the pupal casing, the body is soft and grey in colour and the wings are crumpled. The fly will reach its final stage of maturity by pumping air into its wings and as the exoskeleton dries and hardens, colour appears (Higley & Haskel, 2010).

Whilst this lifecycle is consistent of many fly species, some flies do not always begin by ovipositing eggs. Instead they will sometimes lay live larvae directly onto the carcass. This is known as ovoviviparous behaviour and evident in Sarcophagidae (flesh flies) species, and some species of *calliphorid* flies, i.e. *Calliphora dubia, Calliphora styigia and Calliphora hilli* (Higley & Haskel, 2010). A typical time frame required for the development of a blow-fly egg into a first instar larvae would be between 18-36 hours (Hall & Doisy, 1993; Higley & Haskel, 2010). Blow flies are usually the first insect species to visit a body and in many forensic investigations are known as primary colonisers. In Western Australia the blow flies (Diptera: Calliphoridae), *Lucilia sericata* Meigen 1826*, Calliphora dubia* Macquart 1855*, Calliphora albifrontalis* Malloch 1932 and, *Chrysomya megacephala* Fabricus 1794 have all been previously identified as primary colonisers. While seasonally dependent, *Chrysomya rufifacies* Macquart 1842 and *Chrysomya varipes* Macquart 1841 are usually considered secondary colonisers (Voss et at., 2009).

Since estimations of PMI are based on the rate of development in larvae, it is critical to understanding the complex, individual lifecycles of forensically important flies and the parameters that influence their development before making PMI estimates.

2.4 **The use of insects in post-mortem calculation**

As discussed, when using insects as indicators of mPMI, there are two approaches commonly used. During the early post mortem period, mPMI estimates are based on the development of immature stages of larvae (Henßge et at., 1995). This is calculated from the age of the oldest immature insect present on a corpse (Henßge et at., 1995; Henβge & Madea, 2004). Various mathematical models have been developed around this theory (Grassberger & Reiter, 2001; Grassberger & Reiter, 2002; Higley & Haskel, 2010). The second, during late post mortem interval when the first colonisers have left the body, is based on estimates of the composition of arthropod community, and relates to estimated successional patterns (Catts & Goff, 1992; Larkin et at., 2010). Both these approaches are complementary, although measuring insect development requires larvae are still present on the body.

The time at which the first wave of colonization occurs is the minimum time that body was exposed to insect activity. Therefore, an estimate of the time at which eggs or larvae were deposited represents a minimum estimate of time of death or mPMI although actual death may occur sometime before colonization. The maximum mPMI is an estimate using knowledge of the time the person was last seen alive (Morris & Dadour, 2005; Amendt et at., 2007). It is crucial that investigators are aware of the fact that the period of insect activity on a body does not always correspond exactly with the point of death.

Measuring insect development is a powerful tool for providing estimates of mPMI but many considerations and potential limitations exist when forming an estimate (Higley & Haskel, 2010). A key consideration when calculating insect development is that growth is dependent on temperature. Insects are poikilothermic organisms, and cannot regulate their body temperature. Growth depends on temperature because the biological reactions that form the basis for growth are temperature dependent (Grassberger & Reiter, 2002). As insect growth is temperature dependent it is important to know the temperature at which they were developing for calculations of mPMI. Insects will stop developing below what is known as a lower temperature threshold, and insect growth will also cease at an upper temperature threshold. The lowest temperature at which development can proceed is called the developmental minimum (or minimum threshold), and the highest temperature is called the

developmental maximum (or maximum threshold). The developmental maximum and minimum temperatures vary for different species. It has been suggested the maximum temperature for larval development of *Calliphora* species is 39 °C, whereas *Phormia* species maximum temperature threshold can reach 45 °C (Slone & Gruner, 2007). Developmental max can often be overlooked by entomologists, as temperatures do not normally exceed the highest cut off point, however, in many parts of the world, some days can reach ≥ 40 °C.

The rate of development in immature stages is a function of the ambient temperature surrounding them. It is this theory that forms the basis for age determination of collected specimens, and is known as accumulated degree hours (ADH), or accumulated degree days (ADD) calculations (Goff et al., 1991; Larkin et al., 2010). The technique is based on the hypothesis that between the developmental minimum and maximum temperatures, insect growth is linear in relation to temperature increase. Thus, calculating the age of specimens can be estimated from the summed thermal input they accumulate during growth. ADH and ADD can be calculated by the following formulas (Snyder Sachs, 2001): Time(hours) x (Known Temperature – developmental minimum temp.) = ADH

Time(days) x (Known Temperature – developmental minimum temp.) = ADD

The ADHs or ADDs that are required for certain species to reach a specific developmental stage have been determined by experimentation (Anderson, 2000; Grassberger & Reiter, 2001; Grassberger & Reiter, 2002). As temperatures from the crime scene are usually unknown prior to discovery, an estimation based on information from the nearest weather station is required (Amendt et at., 2007). A mPMI estimate can be formed using ADH or ADD calculations by substituting the temperature conditions at the crime scene into the equation and calculating how long it would have taken under these conditions for the immature larvae to develop to the point they were collected (Anderson, 2000).

Another way of estimating mPMI using development rates of insects is by using isomegalen and isomorphen diagrams (Grassberger & Reiter, 2001; Amendt et at., 2007).

These illustrate morphological changes in fly development depending on time and temperature. An isomorphen diagram is a scatter plot which details the time from eggs hatching until eclosion against temperature. Errors bars on the diagram provide a 95% confidence interval for development stages. An isomegalen diagram plots larval size (length, weigth or width) against temperature (Harvey et. al. 2016). First introduced by Reiter in 1984, and later refined by Grassberger and Reiter in 2001, the diagrams illustrate morphological changes in the development of the fly depending on time and temperature. If the temperature of a crime scene has been considerably constant, as if the case for many corpses found indoors, the age of the maggot can be determined relatively quickly by reading it directly off the diagrams. Grassberger and Reiter (2001), recommend care be taken when using isomegalen and isomorphen diagrams to predict development of flies subjected to changing temperatures, since biological systems under field conditions are rarely predictable. This is why using ADD is a more preferred calculation as it takes into account fluctuating temperatures over days (Grassberger & Reiter, 2001).

Both methods of calculating mPMI rely on correct identification of specimens. Many manuals and procedural guides in forensic entomology clearly outline how to rear collected insect material to adult hood for easier identification (Wallman, 2002; Goff, 2010). It is important to note that fly species of forensic importance will differ from location to location. In Australia, species found to be of forensic importance mainly belong to three genera within the Calliphoridae family: *Calliphora*, *Lucilia*, and *Chrysomya* (Wallman, 2001; Voss et at., 2008; Voss et at., 2009; Cook & Dadour, 2011). A descriptive key is available for species identification of third instar larval and adult stages of the Calliphoridae species within Australia (Wallman, 2001; Wallman, 2000b).

Several abiotic and biotic factors influence the way in which a body decomposes, the associated number of insects, the sequence in which insects arrive/depart, and the number of taxa present (Rodrigues & Bass, 1982: Reibe & Madea, 2010; Voss et at., 2011). These include, geographic location (Campobasso et at., 2001), climate (Shean et at., 1993), seasonal changes (Matuszeweski et at., 2008), physical state (e.g. burnt) (Avila & Goff 1998), and decomposition environment (e.g. in water,

indoors, buried) (Centeno et at., 2002; VanLaerhoven & Anderson, 1999). It is also thought that dietary preference, predation and competition factors also play a role in successional patterns (Fiene et at., 2014). Understanding these parameters is essential when making accurate mPMI calculations with researchers often cautioning others of applying their regionally specific classification system or general schedule of progression to any other geographic area (even if closely related), as it may result in erroneous estimates of mPMI (Galloway, 1997; Cockle, 2013).

As mPMI calculations are based primarily on the development, behaviour and growth patterns of insects, understanding their biology and successional patterns in different environmental conditions is critical to the application of calculating reliable mPMI estimates (Haskel & Catts, 2008). As almost, if not every forensic entomology manual stresses the importance of these factors and their influence on the life of insects, and it is important that this data be available to practitioners (Smith, 1986; Amendt et at., 2010; Byrd & Castner, 2010).

2.5 Factors Influencing Insect Succession and Decomposition

2.5.1 Geographic region

One of the most important factors influencing insect succession and decomposition is the geographic region in which remains are found (Campobasso et al., 2001). The geographic region significantly influences the habitat, vegetation, soil type and weather conditions of that given specific area (Villet & Amendt, 2011). This has a major effect of the types and species of insects present in the area, as well as their seasonal availability (Forbes & Dadour, 2010). Furthermore, geographic location can have a significant effect on decomposition rates which directly impacts the colonization of insect onto the carcass (Higley & Haskell, 2010l; Villet & Amendt, 2011).

Worldwide the distribution for families of carrion insects is relatively ubiquitous, although individual species associated with decomposition can vary significantly between different regions. Reed (1958) identified several variations amongst different species across a wide range of geographic locations. Many of these species

are cosmopolitan, although the same species found in different regions can exhibit unique behavioural patterns that are distinct to that region. For example, throughout most of its distribution the blowfly, *Ch. rufifacies*, is known as a secondary coloniser species often laying eggs during the decay stage of decomposition (Wells & Greenberg 1992, Voss et al., 2009) but in some areas of Australia it has been reported as a primary coloniser of the fresh stage of decomposition (O'Flynn & Moorehouse, 1979). Such behaviours, if not known for a specific geographic areas could significantly affect mPMI estimates during early stage decomposition if entomology data were applied from different geographic locations.

The way a body decomposes can also vary a great deal under different environmental conditions and locations (for example, a dry sandy arid location compared to a wet, tropical location). Combined, the variation in species distribution and the way in which a carcass decomposes inevitably influences the succession rate of insects on and off a carcass in different geographic locations. This is why successional data is often regarded as highly geographically specific (Archer, 2014).

Research conducted in two different forest regions of central Europe by Matuszewski et at., (2010a) identified differences in the colonizing assemblages of flies onto carcasses arising due to seasonal differences. It was also noted that forest type significantly influenced the presence or absence and appearance time of certain taxa. Certain species arrived later on carcasses decomposed in Hornbeam-oak forests compared to the pine-oak forests. Whilst both locations remain geographically close, even minute differences in the habitat within that geographic region can significantly affect the arrival times of insect on and off of a carcass. More apparent changes in habitat within short geographic distances have also been known to effect insect succession (Early & Goff, 1986). For example, in a study conducted on the island of Hawaii, Tullis and Goff (1987) exposed pig carcasses to three different contrasting habitats within the island. Upland forest and woodlands found 1,877 m above sea-level; rainforests found 1,169 m above sea level; and mid-elevation woodlands, 646 m above sea-level. Within each habitat there were considerable differences in the rate of decomposition and insect species composition and successional patters. It is very common for one geographic location to have varying degrees of habitat from coastal areas to inland rural development.

Geographic location has a significant effect on succession patterns, arrival times and species abundance, not only within different countries, but also down to the basic differences of habitat within regions. It is for this very reason that data relating to a specific region detailing the unique insect species and succession processes involved in colonisation must be known to ensure accuracy of prediction based on insects at a crime scene. Due to the nature of crime scenes, and the variability of circumstances surrounding each case, it is critical investigators use relevant, geographic specific data when conducting analysis, or significant errors can occur. While some regions in the world have an appropriate level of reference data on which to draw on (usually areas where forensic researchers reside or are employed), there are still several geographic regions where absolutely no baseline reference data is available to the scientific, and policing community, including several areas within Australia.

2.5.2 Season and Environmental Conditions

Season, along with geographic region, is also considered one of the most influential factors on the rate of decomposition and insect succession of a body (Mann et at., 1990; Archer, 2004a). Change in temperature and environmental conditions\ for any given geographical location directly affects the seasons of an area. This in turn varies the relative humidity, sun exposure and rainfall throughout different times of the year and these conditions are known to have a direct effect on the decomposition rates of a carcass and the presence of insect species, their development rates and abundance (Archer, 2004a; Byrd & Castner, 2010). Insect populations can also wan and peak throughout different seasons in response to climatic conditions. The activity of several insect species can be seasonally specific and certain species may only occur on decomposing remains at specific times of the year (Goff et at., 1988). This is relevant to several forensically important species, and can vastly change the pattern of insect succession within the same geographic area for different times of the year (Wells & Lamotte, 2010).

Additionally, as temperature effects insect growth and activity, warmer temperatures and increased humidity also increase maggot activity, and therefore, increase the rate of decay (Komar, 1998; Matuszewski et at., 2008; Wang et al., 2008; Sharanowski et at., 2008; Matuszewski et at., 2010a). In contrast, cooler

temperatures slow down maggot development and can sometimes inhibit maggot activity altogether (Shean et at., 1993; Sharanowski et at., 2008; Matuszewski et at., 2010a). There is no statistical indication of whether carcasses decay faster when subjected to higher rainfall, although Archer, (2004b) suggests that this would increase the rate of decomposition and Early & Goff, (1983) suggest that the process of decomposition can slow down when exposed to heavy rain fall. Mummification of flesh is also known to have an effect on the rate of flies on and off the body, and is often reported as the product of desiccation. Desiccation is a process that occurs when moisture is rapidly removed from the soft tissues due to climatological conditions. Mummification is often associated with hot and dry climates due to the rapid loss of moisture from a carcass, although this can also occur in very cold climates (Sledzik & Micozzi, 1997; Galloway, 1997).

Seasonal differences can also affect the variety of species present during the year (Benbow et at., 2013). Thus it is important to conduct successional studies over the course of the year to gain an understanding of the presence and absence of species at any given time across the seasons. Typically, succession data pertaining to summer would not be entirely applicable to that derived during winter (Payne, 1965).

2.5.3 Decomposition Environment

The rate of decomposition and insect succession on and off a carcass can vary significantly depending on the environment in which a carcass is decomposing. As a consequence, entomological reference data used to estimate mPMI must be consistent with the environment in which the body was decomposing to ensure the greatest accuracy. Problematically, the number of potential death environments is extensive and thus several gaps remain in the current data available to investigators. Research has focused on the generation of reference data for urban locations (Schumann, 1990); rural environments, including forests and agricultural plantations (Matuszewski et at., 2010a); inside vehicles (Voss et al., 2008); burials (Payne & King, 1968); and water graves (Anderson, 2010b).

Burial is a common method of disposal in criminal cases and clandestine graves are often not very deep, and the top layers of soil usually only provide a simple barrier from colonising insects (Payne et at., 1968). If scavenging animals expose parts of

the body, or the top soil is eroded, insects can still colonise the body, however, arrival time, succession and the decomposition rate of the carcass is often altered (Rodriguez, 1997). During preliminary trials, Rodriguez and Bass (1985) documented that the decomposition of buried bodies occurs a lot slower than that of bodies placed above ground. It was reported that certain fly species could not colonise bodies buried greater than 30 cm, and all insect activity ceased on carcasses buried at greater depths (60cm, and 120cm). Greenberg & Wells, 1998 identified *Megaselia scalaris* (Loew) (Diptera: Phoridae), as an important fly species in relation to buried bodies as it is known to burrow down to bodies buried in coffins and deep in the soil (~180 cm or 6 feet). The flies are small in size which allowes them to travel through the soil and reach buried remains. Calculations of mPMI using *M. scalaris* have been used as evidence in forensic cases (Beneke, et at., 2004).

Aquatic environments present a very unique environmental situation where insect oviposition and colonisation of the body can be compromised (MacDonell & Anderson, 1997; Chin et at., 2008a). A corpse found in water will often go through consecutive stages of sinking and floating. Due to the higher specific weight of a corpse, the body will initially sink, then during the bloat phase, gasses built up in the abdomen will cause it to float to the surface (if not hindered by weights or ligatures tying the carcass down). During this period of floating, if the carcass is within a reasonable distance to the shoreline, airborne insects may colonize the carcass and oviposit in the exposed remains (MacDonell & Anderson, 1997). As the gasses naturally disperse the carcass will eventually sink again. When a body initially sinks, tissues begin to be predated on by natural predators like fish, and other aquatic inhabitants. In a report detailing the submersion of carcasses in a woodland stream, Keiper et al., (1997), detailed the succession of midges (Diptera: Chironomidae) on carcasses. This succession data can be applied to the determination of post-mortem submersion interval in similar cases. Aquatic invertebrates will also colonise a carcass placed in water, including stoneflies and caddisflies species (Merritt & Wallace, 2000). As these species are highly specific to their environment (sea water, river, swimming pool) their identification can indicate movement or transport of a corpse following death.

Blowfly maggots have also been known to remain active and alive within a sunken carcass for several days, and the presence of maggots in the gut and abdominal

regions of a carcass can indicate whether a body has been stored on land for a period of time prior to entering the water, or colonised during the bloat phase (Campobasso et at., 2001). Research surrounding bodies submerged in water is greatly lacking, not only in Australia but worldwide as traditional succession data is not applicable to remains found in water. Prior research indicating species availability and presence or absence in a location can be applied to cases involving water for a given geographic location, although timing and succession data would not be applicable in these cases.

Another environmental scenario which commonly influences decomposition and insect succession is the difference between carcasses found decomposing indoors vs. outdoors. Reibe and Madea (2010) investigated this scenario in Germany. Indoor carcass were exclusively colonised by a single blowfly species (Diptera: Calliphoridae), Calliphora vicina Robineau-Desvoidy 1830 as opposed to five different nacrophagous species of fly which colonised carcasses decomposed outdoors . The indoor carcasses exhibited a significantly lower number of egg batches compared with the outdoor carcasses, and oviposition commonly occurred after 24 hours for the indoor carcasses (Reibe & Madea, 2010). As lower numbers of maggots were present on the carcasses found indoors, large maggot masses did not form, and biomass removal of the carcass was slower compared with the carcasses placed outdoors. This indicates significant differences within the rates of decomposition for carcasses found inside vs. outdoors, and these factors would need to be taken into account for related mPMI estimation.

Voss et al., (2008) compared the difference between carcasses left inside a sealed vehicle (front seat of the car) and carcasses placed exposed, outside on the soil surface. Observed arrival time of primary colonisers were delayed 16-18 hours for the carcasses inside the vehicles, and oviposition did not occur on the carcasses inside the cars until 24-28 hours after primary oviposition had occurred on the carcasses outside. Similarly with carcasses found indoors compared with outdoors (Anderson, 2005a), oviposition times lagged significantly with the carcasses enclosed in the cars. With such great variation in time, considerable error may be introduced into mPMI calculations mPMI were reference data is applied that is not relevant to the death scenario.

The location where a body has been found can help to identify prior movements of the victim or perpetrator. For example, if specific species are found on the body which are known to occur more commonly in urbanised localities and are not indigenous to the location where the body was discovered, this may be a strong indication the death occurred elsewhere, and moved to conceal the crime scene (Erzinclioglu, 1989). Evidence like this could help to speed up an investigation, by indicating post mortem movement of a body, or prior activities in the lead up to the crime, which without entomological evidence may take several days or weeks to discover. Given numerous studies support the importance of matching the details of the crime scene with the decomposition and insect succession data used, ongoing research is needed to ensure the availability of appropriate data for the wide range of common death scenarios forensic investigators encounter

2.5.4 Physical State of Remains

The condition a body is in during decomposition is also an important factor in calculating mPMI (Goff, 2010). Physical state usually relates to the way a body has been killed or disposed of. Trauma inflicted upon a body after death may also alter the state of remains, as in the case of chopping up parts of the body during disposal., The physical state of the body (i.e. covered or bound) during decomposition and any related trauma can add alternate, secondary entry points for insect colonisation or remove natural features like hair and skin. Other physical states can include; burnt (Chin et al., 2008b) hanging (Shalaby et at., 2000); clothed or unclothed (Voss et at., 2011); the presence of drugs (legal/illegal) or toxins (poisons etc.) in the body (Carvalho et at., 2001).

Burning a body is occasionally used by criminals to try and dispose of evidence, or remove a carcass altogether (Anderson, 2005b). Tremendously high temperatures and long incineration times are required to completely dispose of a human body, so much so that recognisable traces of remains are often still present in professional cremation processes (Kennedy, 1996). In the case of criminal activity involving burning, this often leaves bodies only partially burnt allowing decomposition and insect colonisation to proceed, albeit potentially altered. Various studies have concluded that burning alters the attractiveness of fly species to a carcass likely due

to physical changes and/or changes in volatile odour emission (Avila & Goff, 1998; Anderson, 2005b; Chin et at., 2008b).

Voss et al., (2011) compared decomposition and insect succession of clothed and unclothed remains in Perth Western Australia, and concluded that insect arrival and oviposition were mostly consistent between the two treatment groups, although variation occurred in the duration of stay on the body. The black carrion fly (Diptera: Muscidae), *Australophyra rostrata* (Robineau-Desvoidy, 1830), colonised the clothed carcasses at two distinct intervals which lead to a longer larval feeding period on the clothed carcasses compared with the unclothed carcasses. During the later stages of decomposition, where evidence is being collected from successional data in order to make estimates of mPMI, information like this can be critical when making accurate estimates.

Similarly, hanging as a result of either suicide or accident is not an uncommon form of death. If a body is suspended above the ground it provides a unique environment for colonising insects that is not as predominantly influenced by soil dwelling taxa (Chin et at., 2000). This may affect decomposition rate and the associated timing of volatile organic compound release which could impact insect attraction to hanging carcasses. Additionally, the availability of suitable oviposition sites and the ability for soil dwelling taxa to visit the carcass may be altered. As such, insect assemblages may be altered due to the possible exclusion of soil dwelling taxa and feeding conditions. The rate of decomposition may also be affected by the reduction in insect numbers on the body due to larvae falling off and not being about to return (Goff & Lord, 1994).Goff and Lord (1994) noted that hanging altered the drying pattern of the body and excluded soil-dwelling taxa and there was limited dipteran activity overall during the decomposition process. In work on hanging conducted by Shalaby et al., (2000), it was noted that the overall number of different species attracted to the hanging carcasses were lower compared with a carcass placed on the soil surface. Despite the value of these works, which clearly indicate an effect of hanging on decomposition and insect succession, more comprehensive data for hanging death scenarios is still needed. Limitations exist in the scope of the available data in respect to the use of only a single carcass (no replication), appropriate carcass weight, restricted to one geographic region and the absence of broader seasonal representation.

2.6 **Current Issues**

Whilst studies conducted in Western Australia have provided information on baseline reference data for a handful of specific scenarios (Voss et at., 2011; Voss et at., 2008; Voss et al., 2009), significant gaps in the research still exists. Currently, the majority of succession data is based on research conducted over limited time periods and within single seasons. Only one study in Western Australia looks at seasonal differences and this involved guinea pig carcasses rather than the preferred pig model for human decomposition (Voss et al., 2009). Due to the lengthy time periods, cost and logistics often involved in pig carcass research many studies are limited by either low replication or inappropriate animal models.

One death scenario in which limited research has been conducted internationally, as well as locally in Australia, is death and decomposition following hanging. Whilst the generally accepted stages of decomposition were derived from carcasses decomposing laterally on the soil surface (Goff, 2010), only two studies have been published on the effect of hanging on decomposition and insect succession and both studies suffered from low replication (Shalaby et at., 2000; Chin et at., 2010). In particular, no reference data exists documenting hanging specific to the Australasian region. Currently, no reliable data is available for investigators on how decomposition changes with a hanging carcass and/or how this influences insect succession rate and potential calculations for mPMI. The accepted ‘Best practice in forensic entomology – standards and guidelines” (Amedt et at., 2007) makes no mention of how to approach a hanging body or the best place to collect specimens from the body.

2.7 **Hanging in legal investigations**

Hanging is the most common cause of suicide in Australia with the most recently released data showing 56% of all suicide cases involved hanging as a means of death (ABS, 2015). Overall, 1.7% of all deaths in Australia occur from suicide accounting for approximately 1600 deaths a year which require the establishment of mPMI for the coroner, death certificates and peace of mind for the family involved. Whilst most cases of hanging occur inside buildings, direct communication with Australian Federal Police officers in New South Wales indicates that cases of suicide

do occasionally involve scenarios where people have entered the bush, and hung themselves, with officers stating they would attend 2-3 situations like this on a year in NSW, Australia (T. Kelly, personal communication, September 4th, 2014). A video documentary published in 2011 by Vice magazine, titled 'The Aokigahara Forest', discusses how common suicide is in the Aokigahara Forest in Japan. Up to 50-100 deaths occur there annually (Vice, 2016). Bodies remain in the forest for months as authorities only sweep through once annually as the site of the forest is hard to get too and the terrain is difficult to navigate (Vice, 2016).

In cases where bodies are not found for long periods of time, it is often important for families to get closure and understanding surrounding the circumstances, and one of the most important pieces of information available to grieving families can be the time and date of death. Hanging is a death scenario where the body may not be found for several days. For investigators, gathering information about when the person died can help to piece together a time line of events leading to the death and offer evidence in cases of foul play.

2.8 **The effect of hanging on decomposition and insect succession**

Shalaby et al., (2000), suggested that suspension of a carcass above a ground surface increased surface exposure to the air which increased the drying rate of the carcass and therefore increased the decomposition rate. Differences in patterns of drying, exclusion of soil dwelling taxa and gravitational pull are all likely to contribute to differences in decomposition processes between hanging and ground lying carcasses. Gennard (2012) suggested that the natural bacterial processes which occur during decomposition may be increased in hanging carcasses due to the increase in blood transported around the body directly prior to asphyxiation, and this may increase the process of decomposition initially. Some controversy exists, however, in the literature in relation to the cause as other researchers have reported a lag in decomposition in hanging carcasses due to the lack of insect activity directly on the carcass (Goff & Lord, 1994).

Currently two published papers detail the progression of hanging decomposition using juvenile pig carcasses (Shalaby et at., 2000). Carcasses in the study weighed around 10kg each which is considerably lighter than the accepted > 30kg pig model

for human decomposition (Hewadikaram & Goff, 1991). To provide the best model for human decomposition the recommended weight for pig carcasses is 27kg and above (Catts & Goff, 1992; Haskell & Catts, 2008). Recent studies by Matuszewski et al., (2014) conclude that use of pig carcasses be at least 35-40kgs, particularly when first inventories or checklists of forensic succession studies are conducted. The use of juvenile pig carcasses, of ~10kg, in the generation of previous hanging decomposition data (Shalaby et at., 2000; Chin et al., 2010) is thus unlikely to accurately reflect human decomposition (Matuszewski et at., 2014). In the study conducted by Shalaby et at., (2000) one juvenile carcass was suspended by the neck and hung directly from a tree branch using nylon rope and the other was placed laterally on the soil surface 20 metres away (control) from the hanging carcass. The hanging carcass displayed a delayed progression through the physical stages of decomposition which was attributed to a higher rate of drying of the carcass, lack of dipteran larvae establishing masses on the body, and reduced predation of soil dwelling taxa (Shalaby et at., 2000). When maggots fell from the control carcass they were able to regain their position on the body, those on the hanging carcass fell to the ground and were unable to regain access to the carcass. Shalaby et at., (2000) suggested this was the primary reason for lack of maggot masses forming on the hanging carcass, which had a direct effect on the bio mass removal (lagging) in the hanging carcasses compared with the control carcass.

Shalaby et at., (2000) reported that the initial colonisation of both carcasses were similar, with dipteran species ovipositing at common sites like the natural facial orifices, although, differences were observed in the number of taxa present on the hanging carcass (lower) compared with the control carcass. It was also suggested that the ‘drip zone’ (an area that is formed from liquids dripping below the hanging carcass) was more representative of species present on the control carcass as it formed quickly and therefore may more accurately reflect the total period of insect activity then those collected off the body.

Similarly, Chin et at., (2010) conducted a similar experiment using a single 10kg domesticated pig carcass. The animal died of natural causes and was hung using rope from a palm tree in a local palm plantation site. No control, or comparison surface carcass was used during this trial., Much of the information recorded in this publication is based on observations of the number of adult fly species present on

each day and their perceived activity on the carcass and not from collected specimens.

Despite the value of these works, which clearly indicate an effect of hanging on decomposition and insect succession, more comprehensive, geographically specific data for hanging death scenarios is still needed. Limitations exist in the scope of the available data in respect to the use of only a single carcass (no replication), appropriate carcass weight, restricted to one geographic region and the absence of broader seasonal representation. Future work in this area is needed to fill these gaps in the current available research.

2.9 **Conclusion**

Although forensic entomology has been successfully applied to several cases worldwide (Greenberg & Kunich, 1985; Goff, 1993; Goff, 2000), there is still large gaps in the research, especially geographically specific reference data for highly specific cases where bodies have manipulated physical states (i.e burnt, hanging etc.). Lack of baseline studies incorporating the environmental and weather effects of unique death situations can lead to inaccuracy of mPMI estimates. To address this issue, comprehensive decomposition trials under specific conditions will need to be added to current reference data. In regions where there is decomposition and succession data available, studies tend to only provide limited variables outlining basic situations, providing information for a specific death scenario under one set of conditions/habitat/location. Typically, studies conducted historically have insufficient replication and sampling methods which do not meet best practice forensic entomology methods of today.

As forensic entomology is becoming a more understood and popular tool in providing evidence in situations where other traditional methods of providing evidence are unavailable, there is a great need for more highly specific reference data that can be applied to investigations in the future.

Currently, not just in Western Australia, but worldwide, research is yet to fully document the progression of decomposition for hanging carcasses and the associated insect succession. Having no geographically specific reference data for the effect of hanging on bodies could lead to gross inaccuracies and potentially

ineffective mPMI estimations. Thus, baseline analysis of hanging carcass decomposition rates and the associated insect succession onto carcasses is needed in Australia to provide an accurate account and potentially improve the accuracy of mPMI estimates where needed in a real life situation.

Methods and Materials

3.1 Study Site

Decomposition experiments were carried out in three separate trials over a total of three months in 2013. Experiments were conducted within a 253ha wildlife reserve 23 km south of Perth, Western Australia (32° 10'S. 115° 50'E). Within the area where experiments take place, public access is prohibited so the site remains undisturbed between sampling periods. The reserve consists of coastal bushland vegetation, mainly jarrah (*Eucalyptus marginate* Donn ex Smith), marri (*Corymbia calophylla* Lindley) paperbark (*Melaleuca* spp.) and grass trees (*Xanthorrhoea* spp.) (Voss et al., , 2009). Trial 1 was conducted between 19th March - 18th April 2013 (early autumn); trial 2 from the 30th April - 12th of June 2013 (late autumn); trial 3 from the 13th September - 14th October 2013 (spring). Daily temperatures were accessed from the closest weather station located in Jandakot (approximately 8km distance away)(Bureau of Meteorology, 2016).

3.2 Animal Model

In Australia, the use of human remains for field-based research is heavily restricted and prohibited entirely in Western Australia . Animal models, such as dogs, (*Canis lupis familiaris Linnaeus 1758),* rats (*Rattus norvegicus* Berkenhout 1769), guinea pigs (*Cavia porcellus* Linnaeus 1758) and domestic pigs (*S. scrofa*), are the only available option to establish base line reference data on insect succession and decomposition rates in forensically important experiments. Pigs are the preferred model for human decomposition where human bodies are unavailable (Haskel et al., 2002). Previous work has determined negligible difference between human and pig carcasses in respect to insect colonisation patterns (Haskel et al., 2002). As carcass weight is an important factor in hanging due to the elevation of the body having an effect on skin stretching and the body elongating, and pigs bare the closest resemblance to humans in the form of weight and body structure, 50-55kg pigs were selected as animal models. A total of 12 freshly killed pigs were used throughout this project. Four pig carcasses were decomposed in each of three trials (N = 12)

At the beginning of each separate trial, at 10:30 am, four pigs were euthanized by captive head bolt at the Linnely Valley Abattoirs. The carcasses were immediately wrapped in plastic as a measure to prevent insect access prior to the commencement of the trial., Carcasses were transported directly to the study site after being euthanized, with travel time taking approximately 45 minutes. At the study site all carcasses were washed to remove congealed blood. Washing ensured all carcass replicates were of a similar state prior to the commencement of the trial, and blood clots didn't arbitrarily attract insect activity. Two pigs were allocated for hanging (treatment group) and two were decomposed directly on the soil surface (control group). Travel from the abattoir, washing, and hanging process took approx. 2 hours. Trials commenced at 12:30 pm, and this signifies day 1 of the trial.,

3.3 **Experimental set up**

Trials were conducted in flat, semi shaded areas. Control carcasses were placed laterally, directly on the soil surface approximately 15 meters apart. To protect the carcass from animal predation a 1.5m x 2m x .40m steel cage covered with wire mesh was placed over each carcass (Figure 1). The wire mesh was open enough to allow for the free movement of insects on and off the carcass. Cages were lifted during sampling and immediately replaced after each sampling period. To prevent animals digging under the cage to access carcasses, steel sheets (30cm deep) matching the dimensions of the cage were placed vertically into the soil surrounding the carcass. The top of each sheet was flush with the soil surface creating a metal barrier along the sides of the cage beneath the soil surface (Figure 1).

The hanging carcases were hung in a protective steel cage which also provided the structure from which the pigs were suspended. They were located 15 m apart from eachother and the control carcasses. The cage was approximately 3m high x 1.3m wide x 1.3m deep, and covered in steel mesh. The meshing was open enough to allow for the free movement of insects on and off the carcasses. The cage walls were set approximately 30cm beneath the soil surface to prevent animals digging under the cage and gaining access to the carcass. This also stabilised the cage. A hinged door enabled access to the carcasses for sampling (Figure 2).

The pigs were hung using 1.5cm nylon rope threaded through a small steel pulley welded inside the cage. Before the animals were winched off the ground, the rope was tied into a hangman’s knot and positioned tightly under the jawbone of to prevent the animals slipping from the noose. The animals were winched approximately 75cm -1m off the ground.

Figure 1. Representative example of the steel cage used to cover control carcasses to protect against animal predation during the sampling period. Cages were easily lifted for access to the carcass during the trial periods.

Figure 2. A representative example of the steel cage use to protect hanging carcasses from animal predation during the trial periods. The cage had a swing door which acted as an entry point for sampling throughout the trial.,

3.4 **Sampling procedure**

Sampling consisted of daily assessment of decomposition stage and insect collection. The first sampling day was conducted approximately 24 hours after the initial hanging of the carcasses and this signifies day 2 of the trial period. During sampling, prior to lifting the cages or disturbing the carcasses, a brief visual assessment was made which included, assessing the areas where maggots were most predominant. This meant sampling could take place swiftly and with little disturbance to the actual body. Prior to any sampling photographs of each carcass replicate were taken to digitally record the stages of decomposition for later assessment. Five stages of decomposition have been previously defined for pig decomposition; fresh, bloat, decay, post decay and skeletal (Goff, 2010). For the purpose of this study, fresh stage was considered to begin from the moment of death to the onset of bloat. Bloat stage completed with the deflation of the carcass and onset of the decay stage. Escaped body fluids and liquefaction of flesh were present during decay stage. Post decay commenced when the majority of flesh and fluids had ceased, with only dry constitutes, skin, cartilage and bone remaining. The skeletal stage is defined by the absence of remaining skin, with majority of bone exposed with some hair present.

Sampling was conducted between 0900 and 1200h on a daily basis until the carcasses were assessed to be in the late decay stages of decomposition, and maggot activity was becoming reduced on the bodies. This began by day 12-14, there-after, sampling was reduced to every second or third day depending on the observed rate of decomposition. Trial 1 was concluded after 31 days and Trial 2 was concluded after 41 days to increase sampling time as cooler conditions slowed decomposition, and Trial 3 was concluded after 31 days.

Insect samples, including eggs, larvae and pupae were collected where present from various locations on the carcass. Insects were sampled using forceps, spoons and fine tip paint brushes. Roughly 100 maggots were taken in each sample from various locations on the body. Specimens were placed in ventilated containers and labelled with the respective date, time, replicate number and collection site. Specimen containers were stored at 6 $\pm$ 2°C in an esky (ice box) and transported to the laboratory for processing.

Samples were processed immediately after arriving to the laboratory (approximately half an hour drive). During processing, samples were divided into two sub-samples. One was preserved and the remaining sub-sample was reared to adulthood for identification. For preservation, larvae were killed using 80 °C hot water and placed in 70% ethanol for preservation. Pupae which were pricked with a small pin to allow the ethanol into the casing and eggs were placed directly into 70% ethanol.

A record of maggot instar stage was taken from each of the collected samples, by measuring the maggots and recording the number of spiracles present at the tip of the abdomen using a binocular microscope with an ocular eye piece for measuring.

For the purpose of identification collected specimens from each sample were reared through to adulthood using rearing containers. These consisted of a plastic container with 1-2cm of sand placed in the bottom of the containers and a small foam tray resting on top of the sand. The foam tray held a small amount of meat substrate for the larvae to feed on until the larvae moved off the tray into the sand below to pupate. Containers were closed with a mesh lid to prevent escape and foreign insect contamination. The containers were left in a temperature controlled room (24°C) with the lights automatically turned off to simulate night time on a 12 hour rotation. Adult fly emergences were monitored and collected daily, until all adults finished emerging. Adult flies were identified based on publish keys (Wallman 2001a; 2001b) with the aid of laboratory reference specimens identified by the Department of Food and Agriculture, Western Australia.

Results were compiled in tables using Microsoft Excel. Successional tables were created to establish a timeline of insect species arriving upon and leaving the body. This data was compared with weather data for the trial period. Photographs of the decomposition process for each replicate were analysed and changes in decomposition stages recorded and presented in tables alongside the succession information. Insect assemblages for each trial were compared for differences in species activity on the hanging and control carcasses.

3.5 **Weather Conditions**

3.5.1 Trial 1, 19th March – 18th April 2013

Average daily temperature throughout Trial 1 was 21.7 ± 3.1°C with an average maximum temperature of 28.3 ± 4.1°C and average minimum temperature of 15 ± 3.8°C (Figure 3). The overall weather conditions of the 31 day autumn trial (Mar-Apr) were warm and sunny and largely consistent with the late stages of an Australian summer (Jan-Feb). During the beginning of the trial the weather remained consistently warm with temperatures ranging in the mid to high 20s. There was an increase in cloud cover and a total of 25 ml of rain fell over the nights of the 7th and 8th day of the trial., Temperatures dropped to their lowest during this period, with a recorded low of 5.3°C in the early morning directly preceding the recorded showers. Within a 24 hr period of the rainfall occurring, temperatures again reached in their mid to high 20s. The highest daily temperature recorded was 37°C on day 21 of the trial., During the days leading up to this very hot day, the relative humidity dropped to low 30s, and this was indicative of very dry, warm days. Again, there was light scattered rainfall over three days towards the end of the trial which increased the relative humidity at the time and saw a slight fall in temperatures.

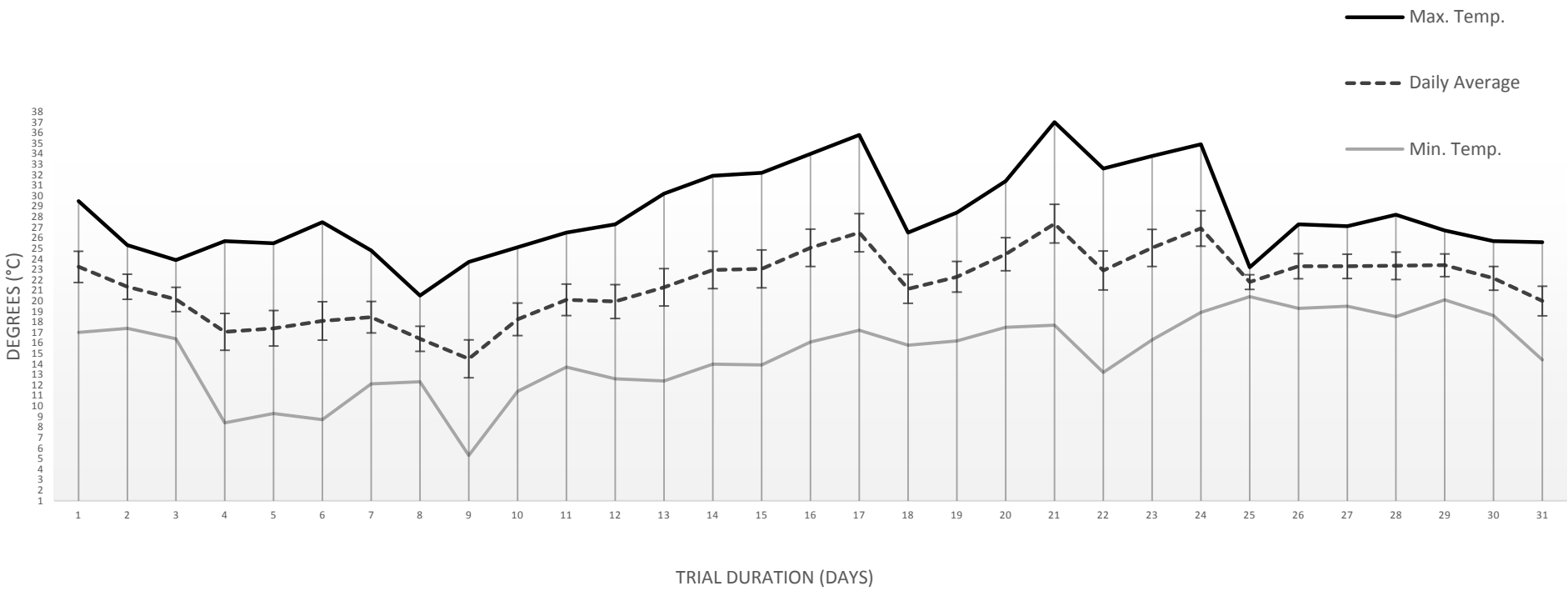


Figure 3. Daily maximum, minimum and average temperatures recorded for Trial 1 over 31 sampling days from March 19th – April 18th 2013.

3.5.2 Trial 2, 30th April – 12th June 2013

Average daily temperature throughout Trial 2 was 14.9°C ± 2.6°C with an average maximum temperature of 21.1°C ± 2.6°C and an average minimum temperature of 8.8 ± 3.8°C°C (Figure 4). The highest maximum temperature of 29.5°C occurred on day 1 of the trial and the lowest daily maximum temperature occurred on day 31 with a high of 14.3°C.

For the time of year, the weather conditions during this trial were slightly irregular due to lengthy periods of rainfall. Over the 41 day trial period there were 5 rain events resulting in 161.0 ml of precipitation (7). The first occurred on the third day of the trial and lasted two days with a total of 18.8 ml of rain falling during this period. The second rain event began on day 9, and lasted four days, with heavy falls totalling 91.6 ml. The third rain event was less significant, with light showers on day 19 and 21, totalling 5.8 ml over the two day period. The fourth rain event began on day 28 and lasted five days, with a total of 39 ml of rain, across the five days. The fifth rain event began on day 37 of the trial, and saw a total of 5.8 ml of rain fall over two days.

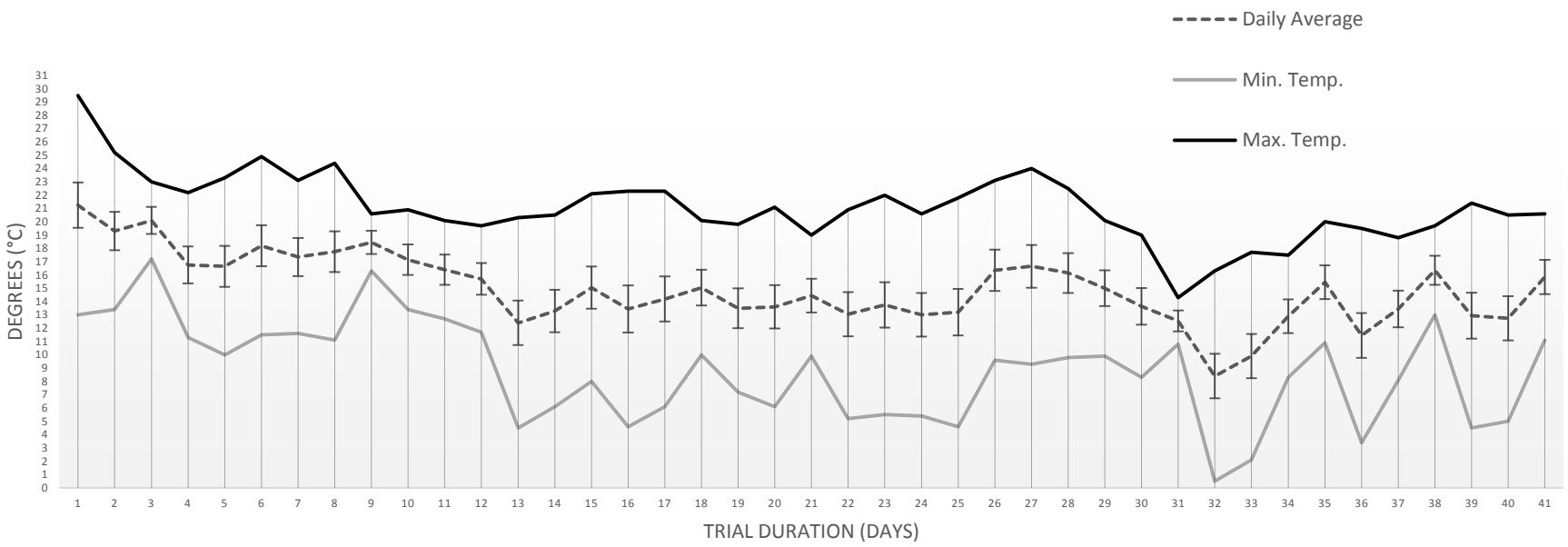


Figure 4. Daily maximum, minimum and average temperatures recorded for Trial 2 over 41 sampling days from 30th April 2013 – 12th June 2013.

3.5.3 Trial 3, 13th September – 14th October 2013

An average temperature of 15.3°C ± 1.8°C occurred throughout Trial 3 with a maximum average of 20.3 ± 2.2°C and an average minimum of 10.4°C ± 2.8°C (Figure 5). The highest recorded temperature was on day 21 with 27.2°C and the lowest recorded temperature was on day 19 with 3.1°C. The overall weather conditions for this trial were very wet, which was unusual for a Western Australian Spring season (usual average 11.7 - 23°C, Australian tourism, 2016). Of the 31 days recorded during this trial period, 10 were rain free and these were scattered throughout the trial which resulted in minimal periods of time for the ground to dry out. Rain fell on the first 13 days of the trial, with 103.2 ml of precipitation recorded. Day 14 saw a break in the rain before sporadic on and off rainfall until the end of the trial, Overall there was a total of 147.2 ml of rain recorded for the 31 day trial period.

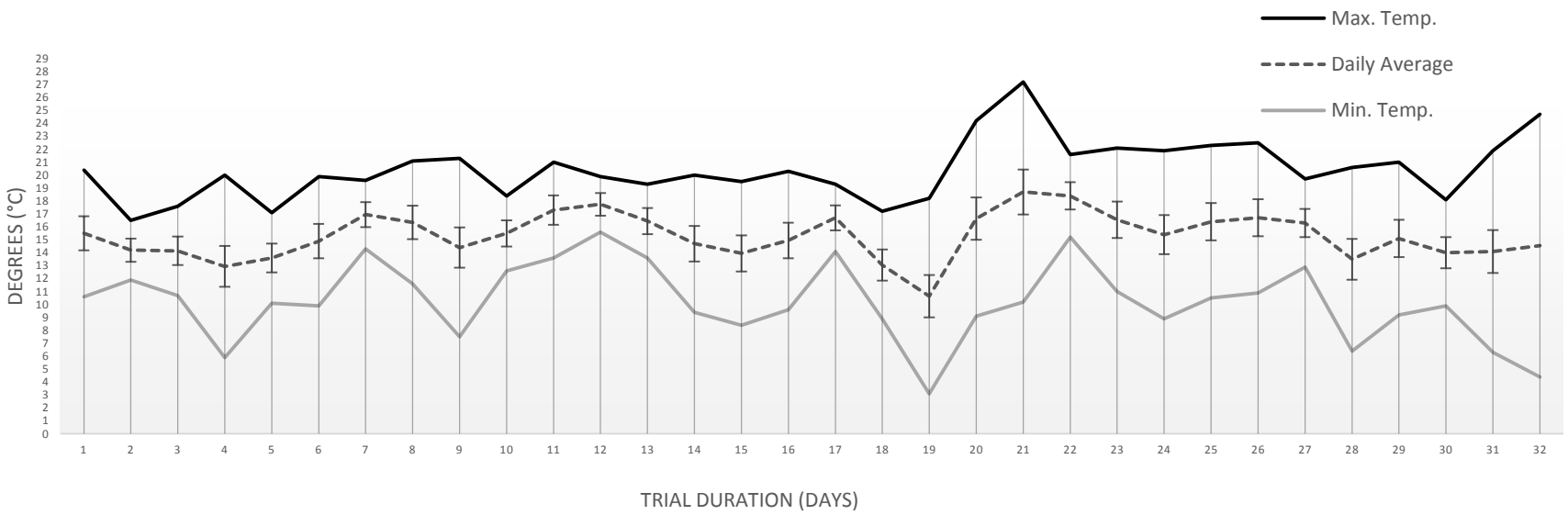


Figure 5. Daily maximum, minimum and average temperatures recorded for Trial 3 over 31 sampling days from September 13th – October 14th 2013.

3.5.4 Weather comparison between trials

Mean ambient temperatures were considerably higher throughout Trial 1 (21.7 ± 3.1°C.) compared with Trial 2 (14.9 ± 2.6°C) and Trial 3 (15.3 ± 1.8°C). This reflects the seasonal timing of the trials with Trial 1 occurring in early autumn where temperatures approximate summer conditions as opposed to the cooler temperatures observed in the latter stages of Trial 2, which occurred in late autumn and progressed into winter. Temperatures throughout Trial 1 and 2 were fairly similar until around day 10 where average daily temperatures were higher during Trial 1 compared to Trial 2. In contrast, cooler temperature conditions occurred right at the start of Trial 3 and temperatures remained comparatively low throughout the trial., After day 10 temperatures were similar between Trial 2 and 3 (Figure 6).

Trial 1 was the driest of the three trials with a total of 29mL of rain failing throughout the entire 31 day trial period and an average relative humidity of 61 ± 18.4 %. In contrast, Trial 2, and Trial 3 were much wetter trials. Trial 2 experienced a total rainfall of 190.6 ml with an average relative humidity of 77.5 ± 13.4%, and Trial 3 experienced a total rainfall of 147.5 ml with an average relative humidity of 69 ± 12.9% (Figure 7).

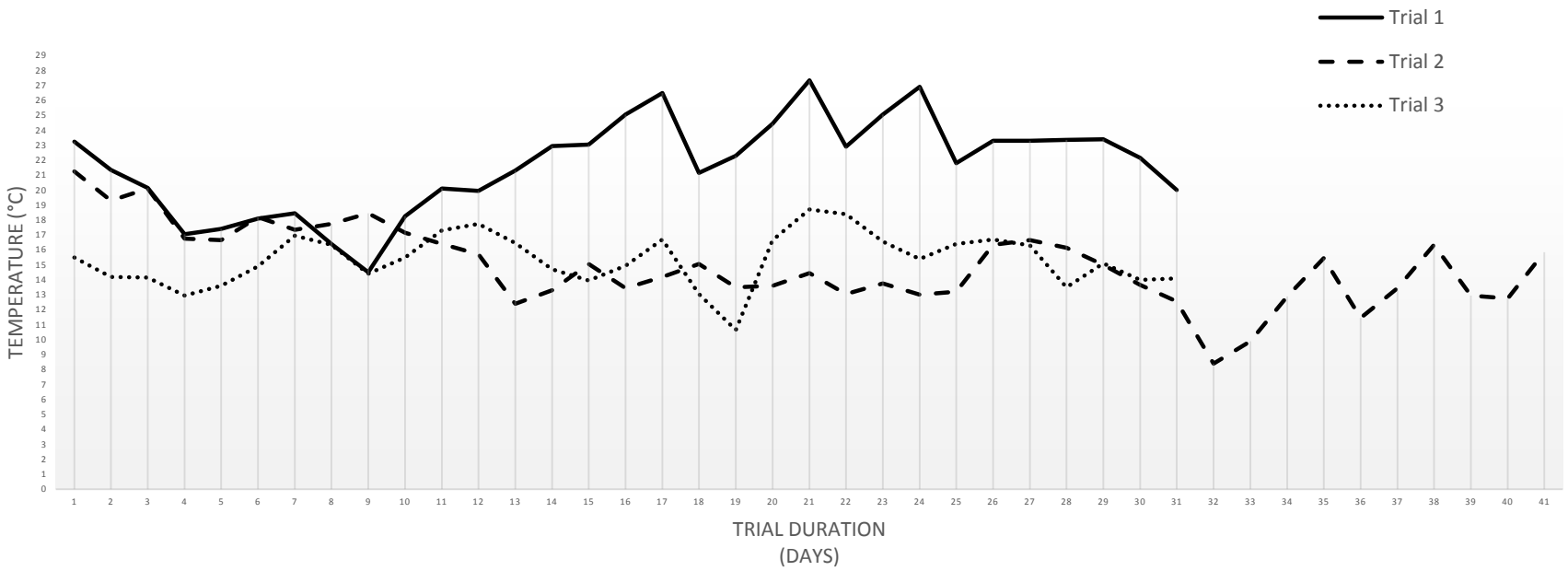


Figure 6. Daily average temperature (°C) observations for three decomposition trials (Trial 1, 19th March – 18th April 2013; Trial 2, 30th April – 12th June 2013; Trial 3, 13th September – 14th October 2013).

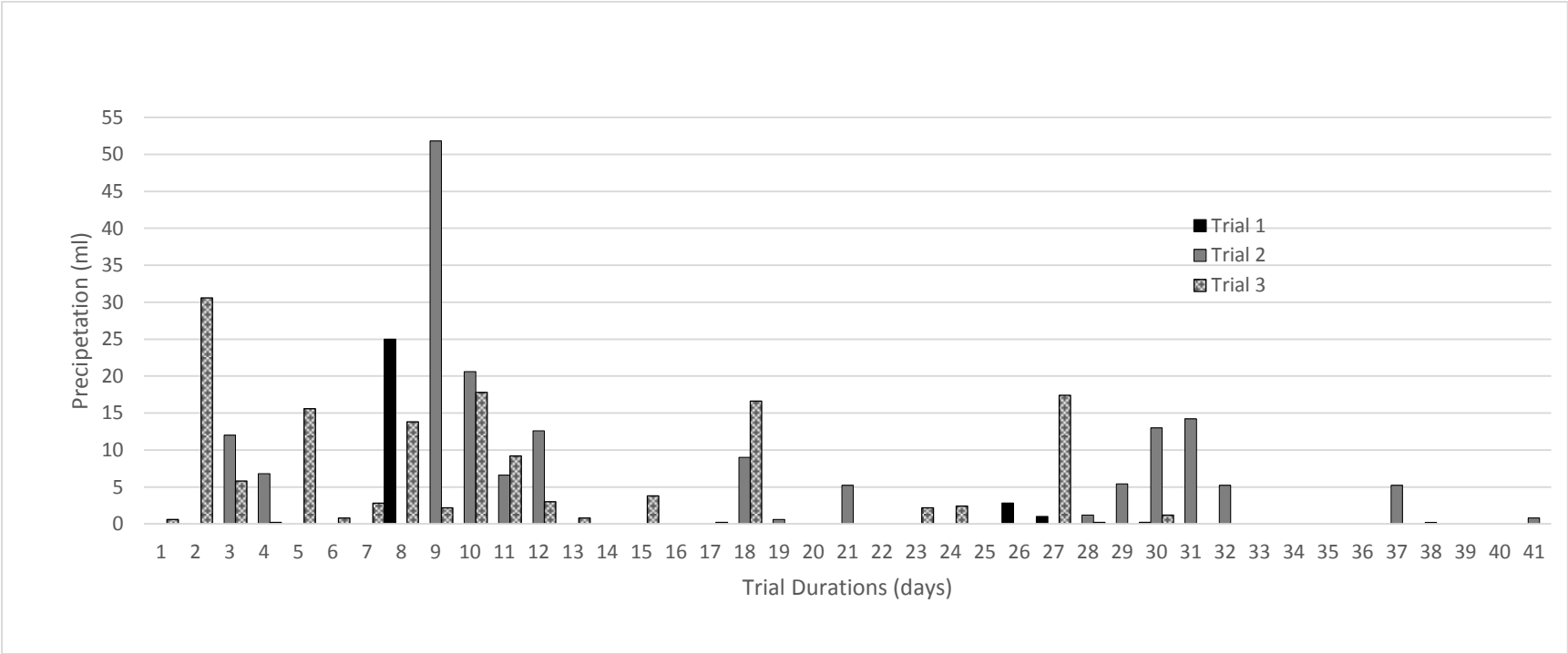


Figure 7. Daily precipitation (ml) throughout the duration of the three trial periods (Trial 1, 19th March – 18th April 2013; Trial 2, 30th April – 12th June 2013; Trial 3, 13th September – 14th October 2013.

Results

4.1 Decomposition

4.1.1 Trial 1, 19th March – 18th April 2013

4.1.1.1 Duration of Decomposition

The control carcasses (carcasses laying laterally on the soil surface) remained in a fresh stage for a very short period of time. They both entered bloat stage within 12 hours and reaching full bloat within 24hrs of death (Figure 8). The duration of bloat varied slightly for both the control carcasses, with one carcass progressing to decay stage by day 4, and the second carcass entering decay by day 6 (Figure 8).

The onset of decay in both carcasses was indicated by skin splitting throughout the abdominal region. In both carcasses, intestines were exposed during this process. Large maggot masses formed along the soil to carcass interface, and by day 11 the entire length of the carcass edge and soil interface of both control carcasses were surrounded by maggot masses. The soil surrounding the carcasses began to take on a blackish colour from maggot activity. The onset of post-decay was indicated by decreased maggot activity, drying of the carcass and loss of biomass in the carcass leaving skin, cartilage and bone remaining. Whilst maggot activity slowed and carcasses ceased to be dominated by feeding activity by day 16 and 17 for Control 1 and 2 respectively (onset of post-decay stage), Control 2, had retained more flesh and cartilage compared with Control 1, but the overall process of decomposition was similar. Both carcasses remained in post-decay for the remainder of the trial.,

Consistent with control decomposition, both hanging carcasses quickly progressed from fresh to bloat within the first 12 hours of death and full bloat was reached within 24hrs of death (Figure 8). The onset of the decay stage was characterised by the abdominal region splitting which in turn, spilt some of the internal contents of both carcasses to the ground. This occurred by day 3, and day 6 for the hanging carcasses. This process created the “drip zone”, an area below the hanging carcasses, formed by seeping fluids and decomposing matter that drops from the hanging carcasses during decomposition. The post-decay stage of decomposition

was reached by day 16, and Hang 2 on day 17. Both hanging carcasses continued in the post-decay stage of decomposition for the remainder of the 31 day trial.

Figure 8. Duration of decomposition stages observed for hanging and control carcasses in Trial 1, 19th March – 18th April 2013.

4.1.1.2 Observed changes in decomposition

Fresh

The fresh stage of decomposition for hanging carcasses was consistent with standard reported observations for control decomposition. Of note, the eyes and mouth on each hanging carcass were slightly open from the pressure of the noose tired around the neck. The limbs hung toward the ground, with the hind limbs closely together (Table 1)

Bloat

As the hanging carcasses reached full bloat, livor mortis occurred in the lower extremities and the greenish discoloration present when the hydrogen sulphide (a significant component of the gasses excreted during decomposition) reacts with the haemoglobin, became evident throughout the lower abdominal region and lower legs (Table 1).

During day 2, the anus on both hanging carcasses became distended and exposed from pressure inside the abdominal region. Liquids began to seep through the skin from the upper chest region and created small pockets of fluids under the upper epithelial tissues. A large maggot mass was present in both hanging carcasses by day 2 and liquid from the mouth also seeped out during the bloat stage. This created a trail of liquid down the chest and abdominal region of the carcass. The tongue in both carcasses was fully extended and swollen within the mouth and the lower legs were extended and pushed slightly outwards.

Decay

Decay was indicated by the onset of abdominal splitting which created the drip zone, explained in further detail below.

During the decay stage of decomposition the hanging carcasses chest and abdomen region began to slowly extend toward the ground lengthening the carcass. The mouth was active with a large maggot mass which likely extended up through the skull cavity and into the ears and nose. Maggots actively fell from the mouth into the drip zone, and liquid continued to seep from the mouth down the chest and abdomen creating a thick yellow residue on the skin. A small mass of maggots formed under

the armpit regions, and maggots were seen crawling in the abdominal cavity. The skin of the hanging carcasses remained largely intact and the anal region continue to extend from the body. The skin became discoloured on the lower legs and under the chin area where blood had pooled and this changed from a green to blackish grey colour throughout the duration of the decay phase. Liquids from the abdominal cavity dripped into the drip zone, with the occasional large mass of internal flesh or bones observed falling into this area.

A maggot mass formed around the anal region and maggots were continually observed dropping from this area into the drip zone. Both carcasses remained in active decay until day 16 and 17 respectively, with the mouth region being the last area of decay activity in both carcasses. Once the mouth and tongue area ceased to actively support maggot masses, and the abdominal cavity ceased to drip liquids into the drip zone, this indicated the onset of post-decay.

Drip Zone

The drip zone was formed when the abdomens of each hanging carcass split open. This created an area below the hanging carcass which acted as an additional site for oviposition and growth of maggots. Throughout the stages of decomposition, the drip zone remained in a similar size and state as it had formed, and a large maggot mass was observed during the decay stage of decomposition. Maggots were observed regularly falling from the hanging carcasses mouth, abdominal cavity and anal area directly into the drip zone.

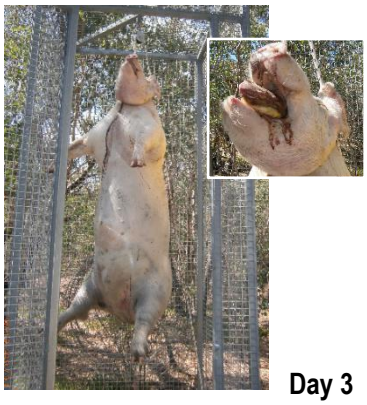
Post-decay

Post-decay in the hanging carcasses was typified by extensive desiccation (Table 1). The abdominal region was stretched and the skin was hard and mummified. The skin from the lower back and lower abdominal regions were blackened from the decomposition process and the upper epithelial layers had dried and flaked off. The head and mouth region on both carcasses showed considerable loss of biomass, but due to hanging the integrity of the carcass remained, although distorted and dried out, which gave the flesh a rich golden colour.

Comparatively, the duration of decomposition stages was consistent between both Hang and Control carcasses and each stage of decomposition was indicated by

similar physical signs for both the hanging and control carcasses throughout the progression of decomposition for Trial 1 (Table 9). At the end of the trial more skin and cartilage remained on the hung carcasses, with a dried leathery appearance throughout the head region and dried flaky skin through the lower back. The abdomen of both hanging carcasses had stretched significantly, increasing the original height of the body. Fly oviposition occurred in the same natural orifices for both the hanging and control carcass i.e. the nose, mouth and anal region. However, duration of insect activity was prolonged in the hanging carcasses, and more skin and cartilage were observed to remain on the hanging bodies compared to the control carcasses.

Table 1. Comparison of decomposition stages between hanging and control carcasses for Trial 1, 19th March – 18th April 2013.

Stage	Hanging	Control
Fresh	 Day 1	 Day 1
Full Bloat	 Day 3	 Day 3
Decay	 Day 7	 Day 8
Post Decay	 Day 15	 Day 16
Skeletal	N/A	N/A

4.1.2 Trial 2, 30th April – 12th June 2013

4.1.2.1 Duration of Decomposition

The onset of the bloat stage occurred on day 3 for the control carcasses (Figure 9). By day 10, one of the control carcasses abdomen split, spilling the internal organs outside of the carcass, indicating the onset of decay. The second control entered decay 2 days later, by day 12 with very limited skin splits along the soil/carcass interface in the abdominal region. The carcass which experienced greater skin splitting throughout the abdominal region, eventually became surrounded by large active maggot masses seen in both controls during Trial 1. On the carcass which did not split, maggot masses remained limited to the head, neck and abdominal area along the soil/carcass interface. The control carcass which split first entered post-decay by day 24, much faster than the second control carcass, which entered post-decay by day 28 of the trial., Both carcasses remained in the post-decay stage of decomposition for the remainder of the trial period.

The onset of the bloat stage for both hanging carcasses occurred by day 4. One of the hanging carcasses remained in bloat stage until day 7, and the second hanging carcass remained in bloat stage until day 9. Neither hanging carcass experienced abdominal splitting. The drip zone below the carcasses began exclusively from liquids seeping from the region onto the soil surface below. A very small drip zone was visible by day 6 for both hanging carcass (4cm diameter) and increased slightly when liquids from the mouth began to seep down the abdomen during decay. The carcasses reached post-decay by day 25 of the trial period, and continued for the remainder of the trial (Figure 9).

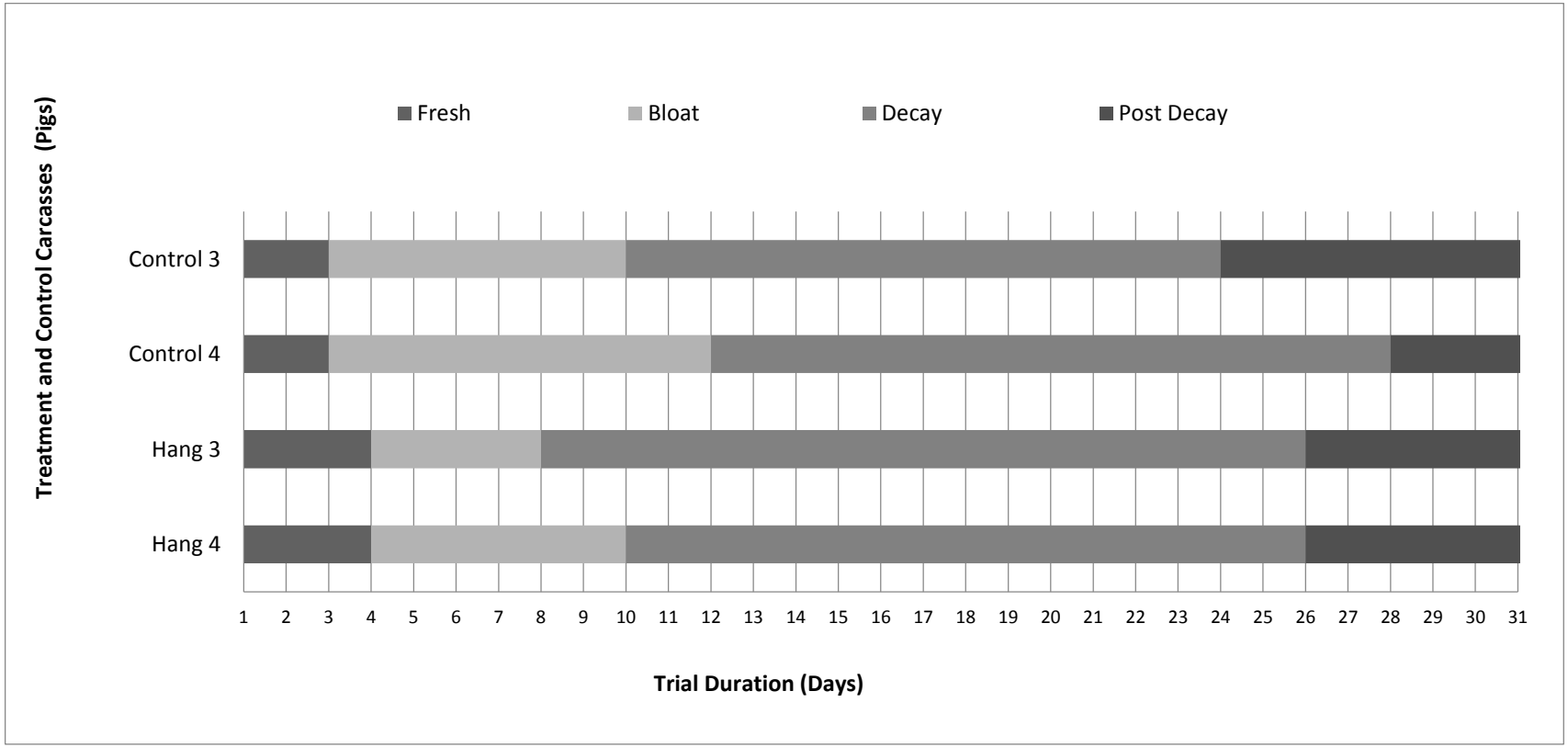


Figure 9. Duration of decomposition stages between hanging and control carcasses in Trial 2, 30th April – 12th June 2013.

4.1.2.2 Observed changes in decomposition

Fresh

Similar to Trial 1 the mouth was slightly open on both carcasses (Table 2). The tongue protruded out to the side of the mouth. The limbs hung toward the ground, with the hind limbs closely together.

Bloat

Full bloat in the hanging carcasses was pronounced and evident throughout the lower abdominal region. Due to the comparatively extended time period it took to reach full bloat for hanging carcasses compared to control, significant blood pooling in the lower legs and lower abdomen area were present. The mouth region on both the hanging carcasses remained partially closed around the protruding tongue, and small maggot masses (1st instar larvae) began appearing in the nostrils and under the tongue which remained in the mouth area.

Drip Zone

A small drip zone began under both of the hanging carcasses on day 6, when liquids began seeping from the anus from pressure build up during the bloat stage of decomposition. Although the carcasses had not reached Decay at this point, elevation of the carcasses and pressure build up in the lower abdominal area caused liquids to seep through the skin whilst in bloat and this is what began a small drip zone area of approximately 6cm in diameter, which grew to 12cm as maggots began to fall from the carcass during Decay. The drip zones for both the hanging carcasses continued to develop as maggots were observed falling from the anus area of the carcasses and collecting in the soil below. This continued for the duration of the trial, but due to neither of the hanging carcasses abdominal areas splitting, the zone area remained small and little nutrients was available for the maggots collecting in this area to develop. Both drip zones remained around 12cm in diameter at their largest.

Decay

The onset of Decay was noticeable when a large active maggot mass was observed extending into the nose and ear cavities with liquification of the tissues in the mouth and cheek areas present. Mummification of the ears, and lower back

began to set in. Lengthening of carcasses throughout the abdominal and chest area was evident during the Decay stage. For both carcasses, the abdomen remained intact (did not split open) and pockets of fluid were observed collecting under the upper epithelial layer in the abdominal area (Table 2). Due to the abdominal areas not splitting in both carcasses, built up gasses remained in this area and the lower abdominal area appeared to remain in a fully bloated state and concurrent processes of decomposition were apparent during this trial.

Post Decay

Maggot activity was concentrated in the head region, therefore the onset of the Post decay in the hanging carcasses occurred when maggot mass activity ceased in this area (Table 2) (and remained inactive in other parts of the carcass). The head region appeared dry and waxy throughout the mouth area, and the back of the head, ears and eye sockets were mummified. Flesh and tissue was retained on the upper and lower limbs on both carcasses and throughout the lower abdominal regions. Lengthening of the abdomen was observed in both of the hanging carcasses, and during post decay this was quite evident as the gasses built up during Bloat stage had slowly dissipated during Decay, and the carcasses both appeared elongated. The flesh in the front abdomen was blackened and signs of mummification were occurring. The carcasses remained in Post decay becoming increasingly dryer for the duration of the trial.,

Comparatively the decomposition stages between the hanging and control carcass in this trial differed considerably. Both the control carcasses abdomens split, but the hanging carcasses abdomens remained intact. This led to varied indicators for the transition from bloat to decay between the treatment groups.

In the hanging carcasses, the head region held the most concentrated amount of maggot activity and therefore played a prominent role in determining the transition from bloat to decay through liquefaction of the mouth region, and activity of maggot masses in this region. When no abdominal splitting occurs, the mouth becomes the predominant indication of decompositional change in a carcass.

The control carcasses rate of decomposition also diverged throughout the trial likely due to an observed increased number of maggot masses formed on one of the

control carcasses compared with the other. This was the most likely reason for the decomposition process to progress much faster than the second control in this trial.,

Lack of abdominal breakage in the hanging carcasses created a concurrent stages of decomposition across the carcasses. Bloat in the lower abdomen and decay in the head mouth and anus regions. The head regions of both hanging carcasses become mummified and waxy in appearance much faster than the lower appendages (Table 2).

Table 2. Comparison of decomposition stages between hanging and control carcasses for Trial 2, 30th April – 12th June 2013

Stage	Hanging	Control
Fresh	 Day 1	 Day 1
Full Bloat	 Day 7	 Day 5
Decay	 Day 12	 Day 12
Post Decay	 Day 24	 Day 24
Skeletal	N/A	N/A

4.1.3 Trial 3, 13th September – 14th October 2013

4.1.3.1 Duration of Decomposition Stages

The onset of bloat began in both control carcasses on day 7, and full bloat was reached by day 13 (Figure 10). The onset of decay began by day 16 of the trial indicated by liquefaction of the mouth region with maggot masses forming in the head region for both control carcasses. Maggot activity was limited throughout the abdominal area, and by day 26, maggot mass activity had ceased and both control carcasses entered into post-decay. Both carcasses had fully deflated by this stage and the head area was partially skeletonised. Decomposition was concentrated to the head and neck region in both control carcasses; therefore timing of events is based on activity in this area.

Comparatively, the onset of bloat began on day 6, with both hanging carcasses reaching full bloat by day 11. By day 13, the onset of decay began with liquefaction of the mouth and larger maggot masses forming the mouth and anal region. Maggot masses in the mouth ceased to remain active after day 24 in one of the hanging carcasses, and day 25 in the other, indicating the onset of post-decay. Similar to the control carcasses, decomposition processes were most concentrated in the head area for the hanging carcasses throughout this trial.,

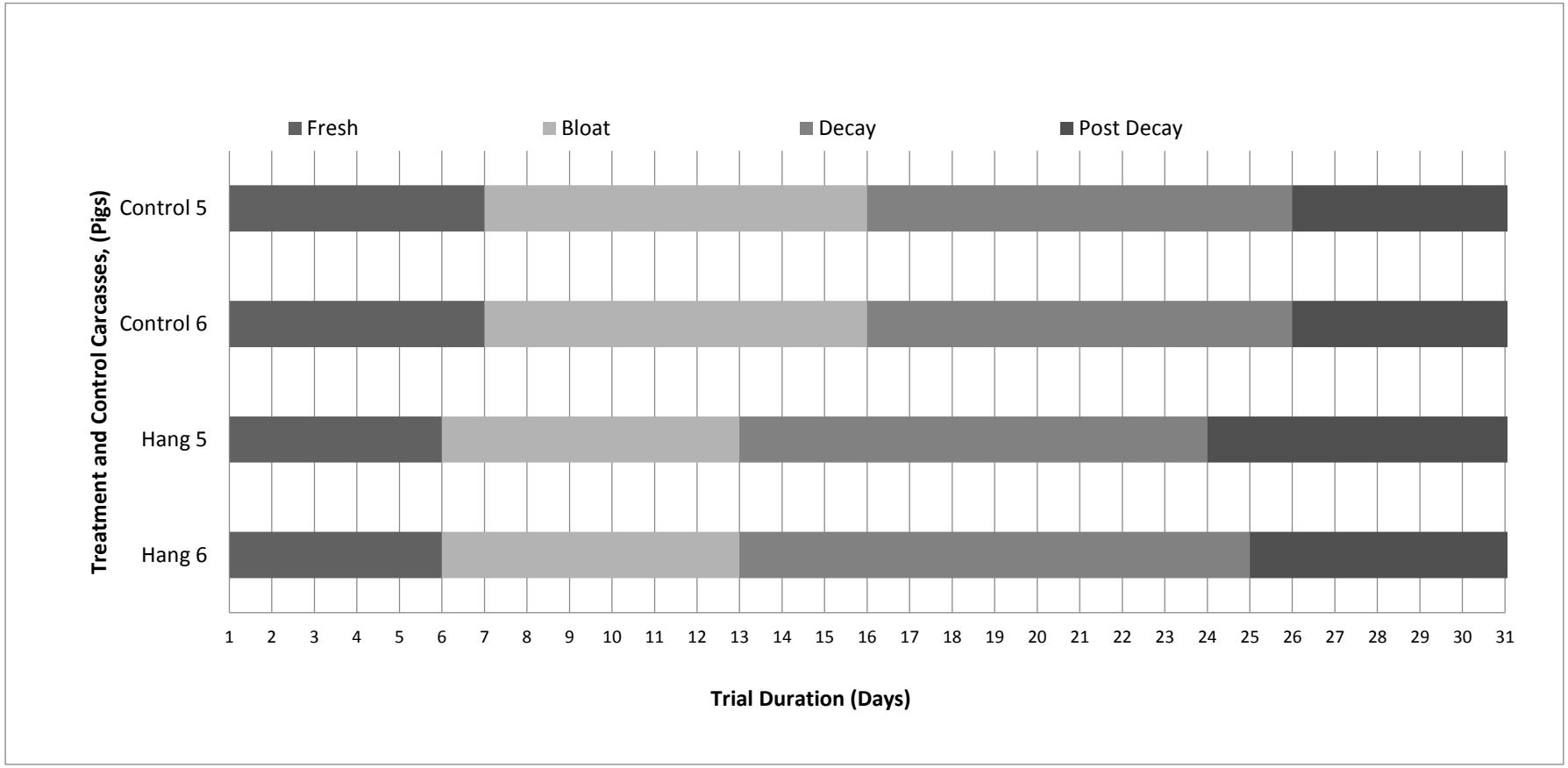


Figure 10. Duration of decomposition stages (days) between hanging and control carcasses in Trial 3, 13th September – 14th October 2013.

4.1.3.2 Observed changes in decomposition.

Fresh

Fresh stage for the hanging pigs was consistent with Trial 1 and 2. Bloating took a longer time to appear in the hanging carcasses during Trial 3, and the carcasses remained in a fresh state with very slight indications of decomposition setting in (green coloration to lower legs) for 2-3 days in the beginning of the trial., Both hanging carcasses began to elongate through the abdominal region before bloat was observed to set in by day 6 (Table 3).

Bloat

Bloat became visible on day 6 of the trial in the hanging carcasses. It then took a further 5 days for the carcasses to reach a fully bloated state (day 11). This was pronounced throughout the lower abdominal region of the carcasses. The anus was slightly extended from the body of the hanging carcasses during the bloat phase (Table 3). In the control carcasses bloating was most prominent throughout the lower abdominal region between the two hind legs. Full bloat was not reached in the control carcasses until day 13 of the trial when the hind legs reached the highest point of extension due to the swelling through the abdominal regions. Maggot masses were concentrated in the mouth and head region

Drip Zone

A small drip zone under each of the hanging pigs began to form on day 11 and 12 for each hanging carcass. The area consisted of a small amount of liquid dripping from the carcass and first and second instar maggots that had been observed falling from the lower abdominal area of each carcass. Similar to Trial 2, in the absence of abdominal splitting, the drip zone has less biomass for actively feeding maggots than that observed in Trial 1.

Decay

The onset of the decay stage in the hanging carcases occurred with the liquefaction and increased maggot mass activity in the mouth region. This occurred on day 12 for both carcasses. During this transition, the lower body of both the carcasses remained in a fully bloated appearance, and skin splitting through the abdomen did

not occur. As decay progressed in the mouth region, the back of the head and ear areas on both carcasses began drying out, and a maggot mass formed along the neck region where the rope was in place. This maggot mass did not establish into a large mass and the masses present on both hanging and control carcasses were limited throughout this trial with masses only observed forming in the head and upper chest/neck region (Table 3).

Post Decay

Post decay was indicated when the maggot mass activity ceased in the head region for the hanging carcasses. This occurred on day 23 and day 24 (Table 3). The head and ear areas were completely mummified. However, due to the lack of abdominal splitting in the hang carcasses during this trial, the abdominal region remains distended and swollen from internal fluid and decomposing flesh build unable to escape the intestinal cavity (Table 3).

Comparatively, decomposition stages during trial 3 were faster in the Hanging carcasses compared with the control carcasses. Maggot activity was concentrated to the head and neck regions for both the control and hanging carcasses throughout this trial and limited maggot masses were observed forming around the abdominal regions where tissues in this area likely formed adipocere, a substance which maggots do not feed on.

The onset of the decay and post decay stages of decomposition were indicated by the progression of decomposition in the head areas in both the control and hanging carcases as this was where the most concentrated amount of decomposition activity occurred in all of the carcasses therefore acted as a clear indication of stages of decomposition.

Table 3. Comparison of decomposition stages between control and hanging carcasses for Trial 3, 13th September – 14th October 2013.

Stage	Hanging	Control
Fresh	 Day 1	 Day 1
Full Bloat	 Day 11	 Day 11
Decay	 Day 17	 Day 17
Post Decay	 Day 23	 Day 25
Skeletal	N/A	N/A

4.2 **Comparison of Decomposition between Trials 1, 2 and 3**

The progression of decomposition was generally similar for the control and hanging pigs within a trial., There was however slight variation between the treatment groups during the two cooler trials where the duration of bloat for hanging pig was shorter than observed for control pigs. Additionally, hanging pigs remained in the post-decay stage longer than control pigs within Trial 2 and 3. When comparing the three trials, there were considerable differences. Trial 1 had the fastest rate of decomposition and carcass appearance during decomposition was considerably different to that of Trials 2 and 3 where temperatures were comparatively cooler.

Trial 1 had clearly defined stages of decomposition with the onset of the decay stage being indicated through the abdominal splitting in both the control and hanging carcasses by day 5 of the trial., This key indicator was not present in Trial 2 with only one of the control pigs experiencing abdominal splitting and neither hanging carcasses abdomens splitting. Abdominal splitting was completely absent in Trial 3, and therefore progression into the decay stage was indicated by other factors like the liquefaction of the mouth and head region. Trial 2 and 3 also varied from Trial 1 with concurrent decomposition processes occurring in the later stages of the trial as bloat remained present in the abdominal areas and the mouth and head progressed into decay. This was more visible in Trial 3, where partial skeletionisation of the head was observed in the control pigs on day 17, as the abdomen retained a large quantity of flesh and adipose tissue had turned into adipocere substance.

The drip zone formation for each trial began at very different times. Trial 1 formed a drip zone within hours of the carcasses being hung. This drip zones began with small amounts of liquid dripping from the carcass and was fully formed following abdominal splitting on day 3 and 5 for the carcasses in Trial 1. A large mass of internal organs fell to the ground and covered an area of approximately 25-30 cm in diameter. Some internal bones also fell into this area, and as decomposition progressed, maggots fell from the mouth and anal regions onto the drip zone below and carried out the remainder of their life cycle in the drip zone area due to the abundance of nutrients in the spilt internal organs. In Trial 2, a drip zone formed solely from liquids dripping from the carcass anal region and became evident on day 6 of the trial for both of the

carcasses. Due to the lack of skin splitting throughout the abdominal cavity, pressure within the lower abdomen caused internal liquid to seeped out from the lower abdominal area quite steadily and maggots began to collect in the drip zone area after falling from the anal area of the carcasses. This created an area approximately 12 cm in diameter. However, lack of abdominal splitting in hanging carcasses resulted in a comparatively smaller drip zone with less nutrients for food supply. Maggot growth was delayed and several maggots were observed dying in this area. Trial 3 was the coolest, wettest month recorded and a drip zone did not form until day 11 and day 12 for each respective hanging carcass. The drip zones were very small, about 10 cm in diameter. Similar to Trial 2, maggots were observed in the soil below the carcass, although the maggots in the drip zone area were not observed to be developing and completing their lifecyle in this area due to the lack of food in the soil.

4.3 **Insect Succession**

Twelve insect taxa, representing four dipteran families were collected and identified in association with carcass decomposition over the duration of the three trial periods (Table 4). Of these, seven species; *Calliphora albifrontalis* (Malloch, 1932), *Calliphora dubia* (Macquart, 1855), *Lucilia sericata* (Meigen, 1826), *Chrysomya megacephala* (Fabricius, 1794), *Chrysomya rufifacies* (Meigen, 1843), *Chrysomya varipes* (Macquart, 1851) and *Australophyra rostrata* (Robineau-Desvoidy, 1830) were consistently observed in all three trial periods. The species *Piophila casei* (Linnaeus, 1758), *Lucilia cuprina* (Wiedemann, 1830) and Sarcophagidae sp. were only evident on carcasses during Trial 1.

Table 4. Dipteran species collected across the three trials. Presence is marked in grey.

H stands for hanging carcasses, C stands for control carcasses. The number represents replicate number for each trial period.

Species List		Trial 1				Trial 2				Trial 3			
Diptera		H1	H2	C1	C2	H3	H4	C3	C4	H5	H6	C5	C6
Family CALLIPHORIDAE	*Calliphora albifrontalis*(Malloch, 1932)	■	■		■	■	■	■	■	■	■	■	■
	Calliphora varifrons (Malloch, 1932)	■									■		
	Calliphora dubia (Macquart, 1855)	■	■	■	■		■	■	■	■	■	■	■
	Lucilia sericata (Meigen, 1826)	■	■	■		■	■	■	■	■	■		■
	Lucilia cuprina (Wiedemann, 1830)		■										
	Chrysomya megacephala (Fabricius, 1794)	■	■	■		■	■	■	■	■			
	Chrysomya rufifacies (Meigen, 1843)	■	■	■	■	■	■	■	■	■	■		■
	Chrysomya varipes (Macquart, 1851)	■	■	■	■	■	■	■	■		■		
MUSCIDAE	*Australophyra rostrata* (Robineau-Desvoidy, 1830)	■	■	■	■	■	■	■	■	■	■	■	■
PIOPHILIDAE	*Piophila casei* (Linnaeus, 1758)			■									
SARCOPHAGIDAE	Sarcophagidae sp.		■	■									

4.3.1 Trial 1 Successional Patterns

4.3.1.1 Control Carcasses

A total of nine species were identified on control carcasses throughout Trial 1 (Table 4, Table 5). Notably five of these taxa, representatives of *L. sericata*, *Ch. megacephala*, Sarcophagidae, *C. albifrontalis* and *P. casei* were only in evidence on one replicate carcass and *C. albifrontalis* and *P. casei*. taxa were infrequently observed. In contrast, the four most active and abundant colonisers of control carcasses were *C. dubia*, *Ch. rufifacies*, *Ch. varipes* and *A. rostrata*.

Calliphora dubia was the first species to colonise control carcasses, collected on day 2 for both the control carcasses (Table 5, Table 6). First instar maggots were found in the nose and head region on day 2 and continued to be observed on control carcasses during sampling until day 6 of the trial.,

Chrysomya rufifacies was the dominant coloniser of both control carcasses arriving on day 4 and in evidence on both carcasses in high numbers until day 25. For one of the control carcasses, both second and third instar *Ch. rufifacies* were still present on the carcass on the last days of the trial, although pupae were first observed on day 15 (Table 5). Shortly after the arrival of *Ch. rufifacies, Ch. varipes* was collected from both carcasses on day 7 and similarly larvae remained present on control carcasses until the end of the trial.,

Consistent with previous reports of insect succession for Western Australia, *A. rostrata* maggots were observed late in the trial (Voss et at., 2009) on day 12 and 15 on each carcass respectively and remained present on the carcases for the duration of the trial (Table 5; Table 6)

While only observed on one control carcass, *L. sericata* and *C. albifrontalis* eggs were collected on day 8 and 9 respectively. Similarly, representatives of the Sarcophagidae sp. and *Ch. megacephla* arrived on day 4 and 5 respectively. Second instar *P. casei* were collected on day 9 from one control carcass and not observed at any other sampling period or on hanging carcasses throughout the trial, similarily with *L. cuprina* was only present on one of the hanging carcasses during this Trial 1.

Table 5. Dipteran occurrence matrix first control carcass Trial 1. Samples were collected daily and records indicate the day and stage of larval growth upon collection: (E) eggs, (1) first instar, (2) second instar, (3) third instar, (P) pupa(e).

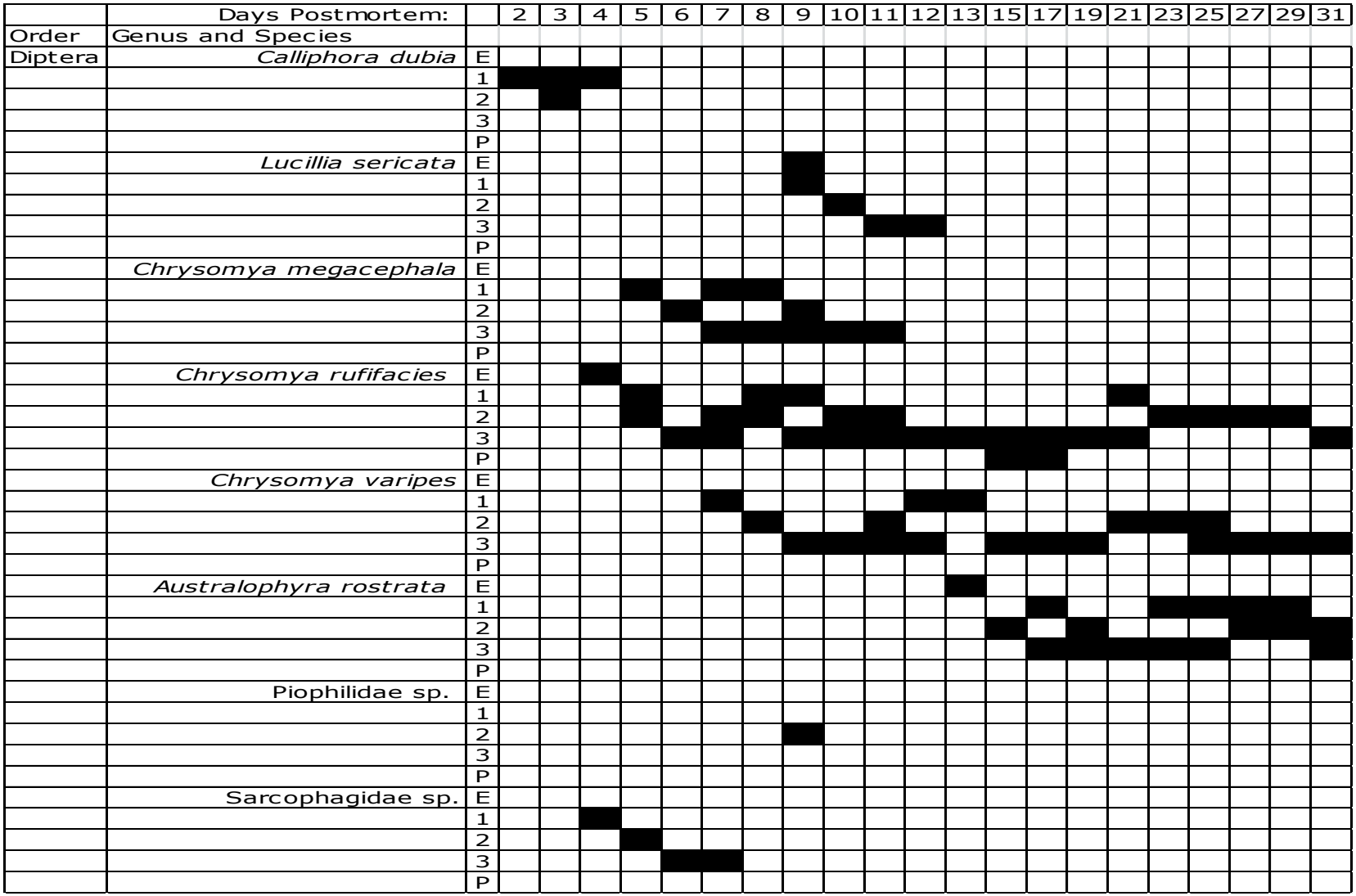


Table 6. Dipteran occurrence matrix second control carcass Trial 1. Samples were collected daily and records indicate the day and stage of larval growth upon collection: (E) eggs, (1) first instar, (2) second instar, (3) third instar, (P) pupa(e).

	Days Postmortem:		2	3	4	5	6	7	8	9	10	11	12	13	15	17	19	21	23	25	27	29	31
Order	Genus and Species																						
Diptera	*Calliphora albifrontalis*	E							■														
		1								■													
		2								■													
		3																					
		P																					
	Calliphora dubia	E																					
		1	■	■		■																	
		2		■	■																		
		3				■																	
		P																					
	Chrysomya rufifacies	E				■																	
		1					■			■													
		2					■	■	■	■		■	■	■									
		3						■	■	■	■	■	■	■	■	■							
		P														■	■	■	■				
	Chrysomya varipes	E																					
		1						■															
		2							■		■	■	■	■				■		■			
		3								■	■	■	■	■	■	■							
		P																					
	Australophyra rostrata	E																					
		1														■		■			■		■
		2											■					■	■		■	■	■
		3													■	■	■	■		■	■	■	■
		P																					

4.3.1.1 Hanging Carcasses

A total of nine species were identified on hanging carcasses throughout Trial 1 with a tenth species collected only from the drip zone beneath hanging carcasses (Table 7, Table 8). In contrast to control succession patterns, the most active and abundant colonisers of hanging carcasses encompassed a wider range of species including *C. albifrontalis*, *C. dubia*, *L. sericata*, *Ch. megacephala*, *Ch. rufifacies* and *Ch. varipes*. Additionally, *A. rostrata,* evident on control carcasses in the later stages of the trial, was never directly collected off the hanging carcasses but was detected beneath hanging carcasses in the drip zone as early as day 8.

Consistent with insect colonisation of control carcasses, *C. dubia* were the first species to conlonise hanging carcasses with first and second instar maggots collected on day 3, suggesting an earlier arrival on day 2. Second instar *C. dubia* maggots were collected from the drip zone on day 6. . The blowfly, *C. dubia* remained present on the carcasses until day 15 when pupa were collected from the mouth of one of the hanging carcasses (Table 8).

Eggs of *C. albifrontalis* were observed on day 7 and 8 on hanging carcasses and eggs were similarly noted in the drip zone on day 8. However, second instar maggots were observed in the drip zone beneath one hanging carcass on day 7 (Table 4). Results are unclear if the second instar maggots fell from the body originally, or were oviposited in the drip zone directly at an earlier time point.

Additionally, *Ch.megacephla* eggs were first detected on hanging carcasses on day 8 and remained active on both carcasses for the duration of the trial., Third instar maggots of the species were detected in the drip zone but only for one of the carcasses. Again it is unclear if oviposition occurred originally on the hanging carcass or in the drip zone itself.

The arrival of *Ch. rufifacies* was comparatively delayed compared to control carcasses with eggs detected on hanging carcasses on day 10 compared to day 4 on control carcasses and day 9 in the drip zone (Table 9). A steady progression from eggs to third instar maggots were collected from both carcasses for the remainder of the trial., On day 21 for one of the hanging carcasses, a new batch of eggs were collected.

Again in contrast to insect succession onto control carcasses, representatives of *C. varipes* were less frequently observed on hanging carcasses and arrival was considerably later with first instar maggots detected on day 12 as opposed to day 7 on control carcasses (Table 9).

Finally, *L .sericata* eggs were noted on one hanging carcass on day 6 as ooposed to day 9 on control carcasses and were more frequently observed throughout the trial in association with hanging carcasses (Table 9). A second succession wave was also detected on day 23 when eggs were again collected off one of the hanging carcasses.

There was no notable pattern of succession onto the drip zone area, and as noted before, several maggots were seen to fall from crowded maggot mass areas like the mouth and the anus region which formed a majority of the maggots in the drip zone. Similar instar levels were collected from the drip zone area and off the carcass at the same time, indicating the majority of the larvae found in the drip zone most likely originated on the carcass. The only exception was the presence of *A. rostrata* which was only collected in the drip zone for both of the hanging carcasses during Trial 1. For one of the hanging carcasses this species was present by day 8 in the drip zone area and remained active for the remainder of the trial, whilst this species was only detected on day 31 as a third instar maggot for the second hanging carcass. Being a third instar indicated that this species had likely been present in the drip zone several days prior to collection

Table 7. Dipteran occurrence matrix first hanging carcass Trial 1. Samples were collected daily, and records indicate the day and stage of larval growth upon collection: (E) eggs, (1) first instar, (2) second instar, (3) third instar, (P) pupa(e). If specimens were collected directly from carcass this is in dicated by the (H) column. If specimens were collected from the drip zone, this is indicated in the (D) column.

	Days Postmortem:		2		3		4		5		6		7		8		9		10		11		12		13		15		17		19		21		23		25		27		29		31	
	Zones		H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D
Order	Genus and Species																																									
Dipera	*Calliphora alibifrontalis*	E																																								
		1																																							
		2																																							
		3																																							
		P																																							
	Calliphora varifrons	E																																							
		1																																							
		2																																							
		3																																							
		P																																							
	Calliphora dubia	E																																							
		1																																							
		2																																							
		3																																							
		P																																							
	Lucillia sericata	E																																							
		1																																							
		2																																							
		3																																							
		P																																							
	Lucillia cuprina	E																																							
		1																																							
		2																																							
		3																																							
		P																																							
	Chrysomya megacephala	E																																							
		1																																							
		2																																							
		3																																							
		P																																							
	Chrysomya rufifacies	E																																							
		1																																							
		2																																							
		3																																							
		P																																							
	Chrysomya varipes	E																																							
		1																																							
		2																																							
		3																																							
		P																																							
	Australophyra rostrata	E																																							
		1																																							
		2																																							
		3																																							
		P																																							

Table 8. Dipteran occurrence matrix second hanging carcass, Trial 1. Samples were collected daily, and records indicate the day and stage of larval growth upon collection: (E) eggs, (1) first instar, (2) second instar, (3) third instar, (P) pupa(e). If specimens were collected directly from carcass this is indicated by the (H) column. If specimens were collected from the drip zone, this is indicated in the (D) column.

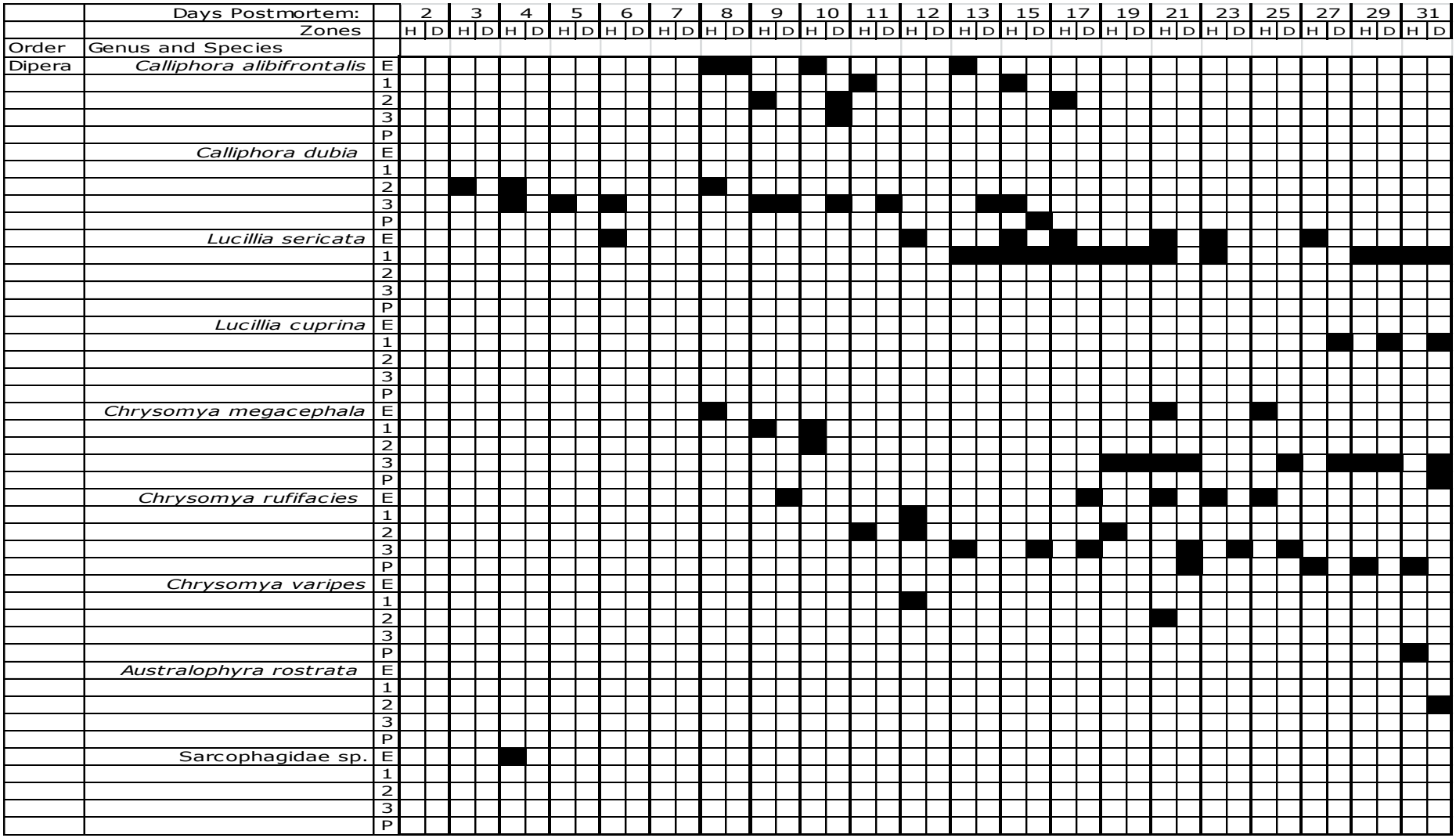


Table 9 Dipteran occurrence matrix for both treatment groups during Trial 1. Source indicates which treatment group the sample was collected including the drip zone.(C) controls; (H) hanging (D) drip zone. The black cell indicates species present on both replicates and the grey cells indicate presence on one replicate only

		Days Postmortem																				
		2	3	4	5	6	7	8	9	10	11	12	13	15	17	19	21	23	25	27	29	31
Species	Source																					
Calliphora dubia	C																					
	H																					
	D																					
Chrysomya rufifacies	C																					
	H																					
	D																					
Sarcophagidae sp.	C																					
	H																					
	D																					
Chrysomya megacephala	C																					
	H																					
	D																					
Lucillia sericata	C																					
	H																					
	D																					
Calliphora albifrontalis	C																					
	H																					
	D																					
Chrysomya varipes	C																					
	H																					
	D																					
Australophyra rostrata	C																					
	H																					
	D																					
Piophila casei	C																					
	H																					
	D																					
Calliphora varifrons	C																					
	H																					
	D																					
Lucillia cuprina	C																					
	H																					
	D																					

4.3.2 Trial 2 Successional Patterns

4.3.2.1 Control Carcasses

Lucillia sericata and *Ch. megacephla* were the primary coloniser species on both control carcasses, being detected on day 4 as first and second instar maggots. This was closely followed by oviposition of *C. albifrontalis*, *Ch. rufifacies* and *Ch. varipes* on day 5. *C. dubia* was not as dominant throughout this trial as in trial 1, being identified as a secondary coloniser species arriving on day 6 on one of the hanging carcasses and day 8 on one of the hanging carcasses.

The colonisation patterns were similar in both of the control carcasses for Trial 2, with the primary colonisers being *L. sericata* and *Ch. megacephla , and C. albifrontalis* remaining on the carcasses until days 13-16 where they were last collected (with the exception of *L. sericata* being identified on day 27*)* and the secondary colonisers, *Ch. rufifacies* and *Ch.varipes* remained dominant on the carcasses for the remainder of the trial.,

A. rostrata was sporadically observed throughout the trial, collected initially on days 4-8 and then again on days 19 and 21 (Table 10, Table 11).

Table 10. Dipteran occurrence matrix, first control carcass Trial 2. Samples were collected daily and records indicate the day and stage of larval growth upon collection: (E) eggs, (1) first instar, (2) second instar, (3) third instar, (P) pupa(e).

	Days Postmortem:		2	3	4	5	6	7	8	9	10	11	12	13	16	17	19	21	23	25	27	29	32	35	38	41
Order	Genus and Species																									
Diptera	*Calliphora albifrontalis*	E																								
		1				■		■	■				■	■												
		2				■	■						■													
		3									■															
		P																								
	Calliphora dubia	E																								
		1							■	■	■															
		2																								
		3																								
		P																								
	Lucillia sericata	E																								
		1			■	■						■	■		■											
		2				■	■																			
		3										■														
		P																								
	Chrysomya megacephala	E																								
		1			■				■	■		■	■	■												
		2			■	■	■																			
		3					■	■	■	■																
		P																								
	Chrysomya rufifacies	E				■	■	■	■																	
		1				■			■		■	■		■	■			■								
		2					■	■		■	■	■	■	■	■	■		■	■				■			
		3								■				■	■	■	■	■	■	■	■	■	■	■	■	■
		P																					■	■		
	Chrysomya varipes	E				■		■	■																	
		1					■						■	■												
		2						■	■	■		■	■	■	■	■		■						■		
		3								■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■
		P																								
	Australophyra rostrata	E						■	■											■				■		
		1													■									■	■	
		2																		■		■	■		■	■
		3																■	■		■	■	■			
		P																								

Table 11. Dipteran occurrence matrix, second control carcass Trial 2. Samples were collected daily and records indicate the day and stage of larval growth upon collection: (E) eggs, (1) first instar, (2) second instar, (3) third instar, (P) pupa(e).

	Days Postmortem:		2	3	4	5	6	7	8	9	10	11	12	13	16	17	19	21	23	25	27	29	32	35	38	41
Order	Genus and Species																									
Diptera	*Calliphora albifrontalis*	E																								
		1																								
		2																								
		3																								
		P																								
	Lucillia sericata	E																								
		1																								
		2																								
		3																								
		P																								
	Chrysomya megacephala	E																								
		1																								
		2																								
		3																								
		P																								
	Chrysomya rufifacies	E																								
		1																								
		2																								
		3																								
		P																								
	Chrysomya varipes	E																								
		1																								
		2																								
		3																								
		P																								
	Australophyra rostrata	E																								
		1																								
		2																								
		3																								
		P																								

4.3.2.2 Hanging carcasses

The species observed on hanging carcasses were similar with those observed on control carcasses throughout Trial 2. Again, *Ch. megacephala* and *L. sericata* were among the initial colonisers of hanging carcases although representatives of *C. albifrontalis* were the very first species to be detected on both hanging carcasses for Trial 2 (Table 12; Table 13). *C. albifrontalis* remained active for nearly the entire trial period (Table 13).

Additionally, *L. sericata* and *Ch.megacephla* continued to be collected off the hanging carcass and drip zone for a majority of the remainder of the trial period.

Overall the hanging carcasses were dominated by *L. sericata, Ch. megacephla, C. albifrontalis*, *Ch. rufifacies* and *Ch. varipes*, with these species ovipositing early in the trial and remaining detectable for the duration of the trial (Table 14). In contrast to Trial 1, *Ch. rufifacies* and *Ch. varipes* arrived earlier on hanging carcasses (day 3 and 4 respectively) compared to control carcasses (day 5). Both species were less abundant on hanging carcasses than on control carcasses. Within Trial 2, the arrival of species was consistent across treatments (Table 14). The one exception was the earlier arrival of *A. rostrata* on control carcasses (day 4) compared to hanging carcasses (day 15). The species was collected directly from the hanging carcasses as well as the drip zone on days 25 and 27.

Table 12. Dipteran occurrence matrix, first hanging carcass Trial 2. Samples were collected daily, and records indicate the day and stage of larval growth upon collection: (E) eggs, (1) first instar, (2) second instar, (3) third instar, (P) pupa(e). If specimens were collected directly from carcass this is indicated by the (H) column. If specimens were collected from the drip zone, this is indicated in the (D) column.

	Days Postmortem:		2		3		4		5		6		7		8		9		10		11		12		13		15		17		19		21		23		25		27		29		32		35		38		41	
	Zones		H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D
Order	Genus and Species																																															
Dipera	*Calliphora alibifrontalis*	E																																														
		1																																														
		2																																														
		3																																														
		P																																														
	Lucillia sericata	E																																														
		1																																														
		2																																														
		3																																														
		P																																														
	Chrysomya megacephala	E																																														
		1																																														
		2																																														
		3																																														
		P																																														
	Chrysomya rufifacies	E																																														
		1																																														
		2																																														
		3																																														
		P																																														
	Chrysomya varipes	E																																														
		1																																														
		2																																														
		3																																														
		P																																														
	Australophyra rostrata	E																																														
		1																																														
		2																																														
		3																																														
		P																																														

Table 13. Dipteran occurrence matrix, second hanging carcass Trial 2. Samples were collected daily, and records indicate the day and stage of larval growth upon collection: (E) eggs, (1) first instar, (2) second instar, (3) third instar, (P) pupa(e). If specimens were collected directly from carcass this is indicated by the (H) column. If specimens were collected from the drip zone, this is indicated in the (D) column.

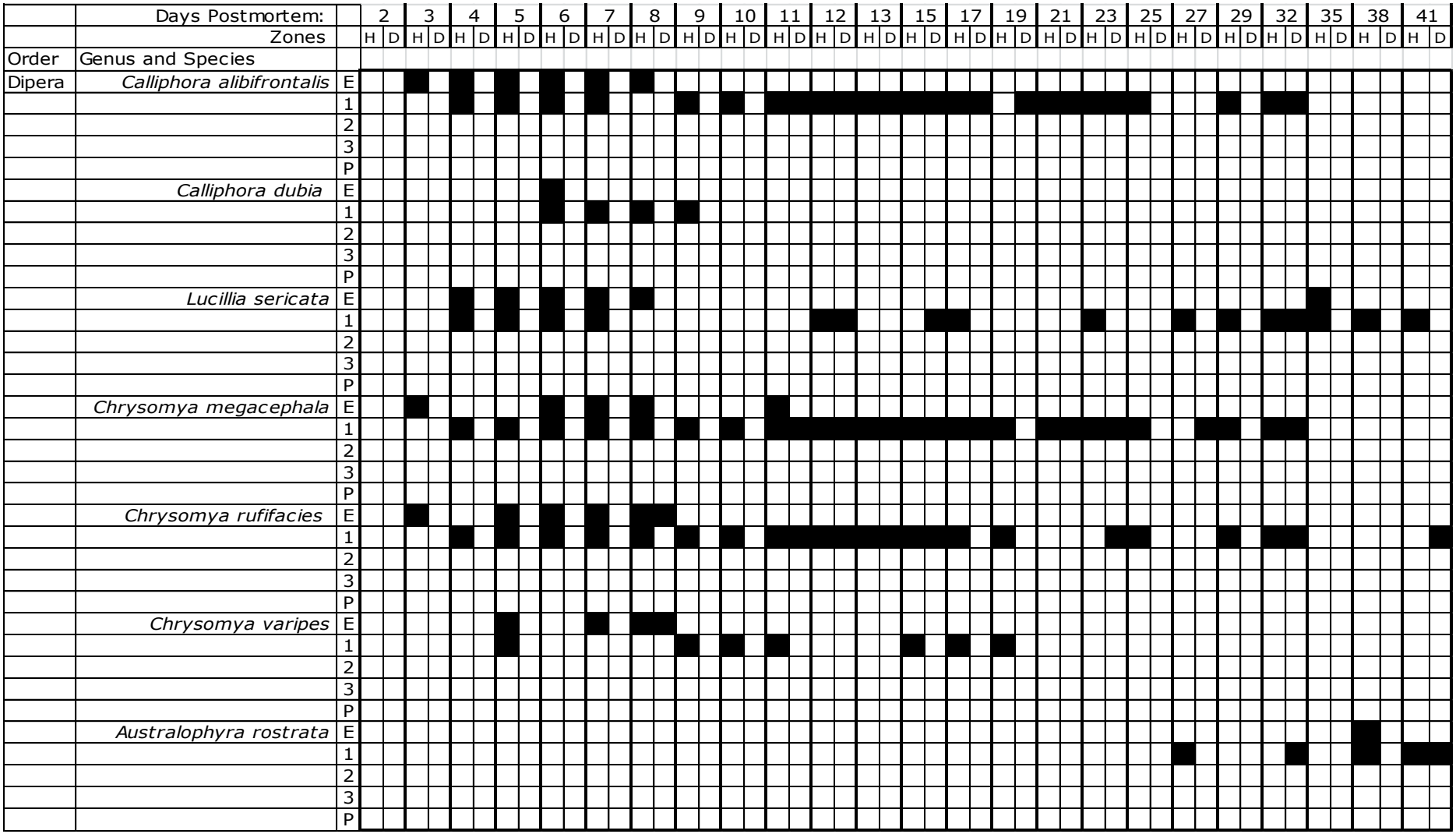
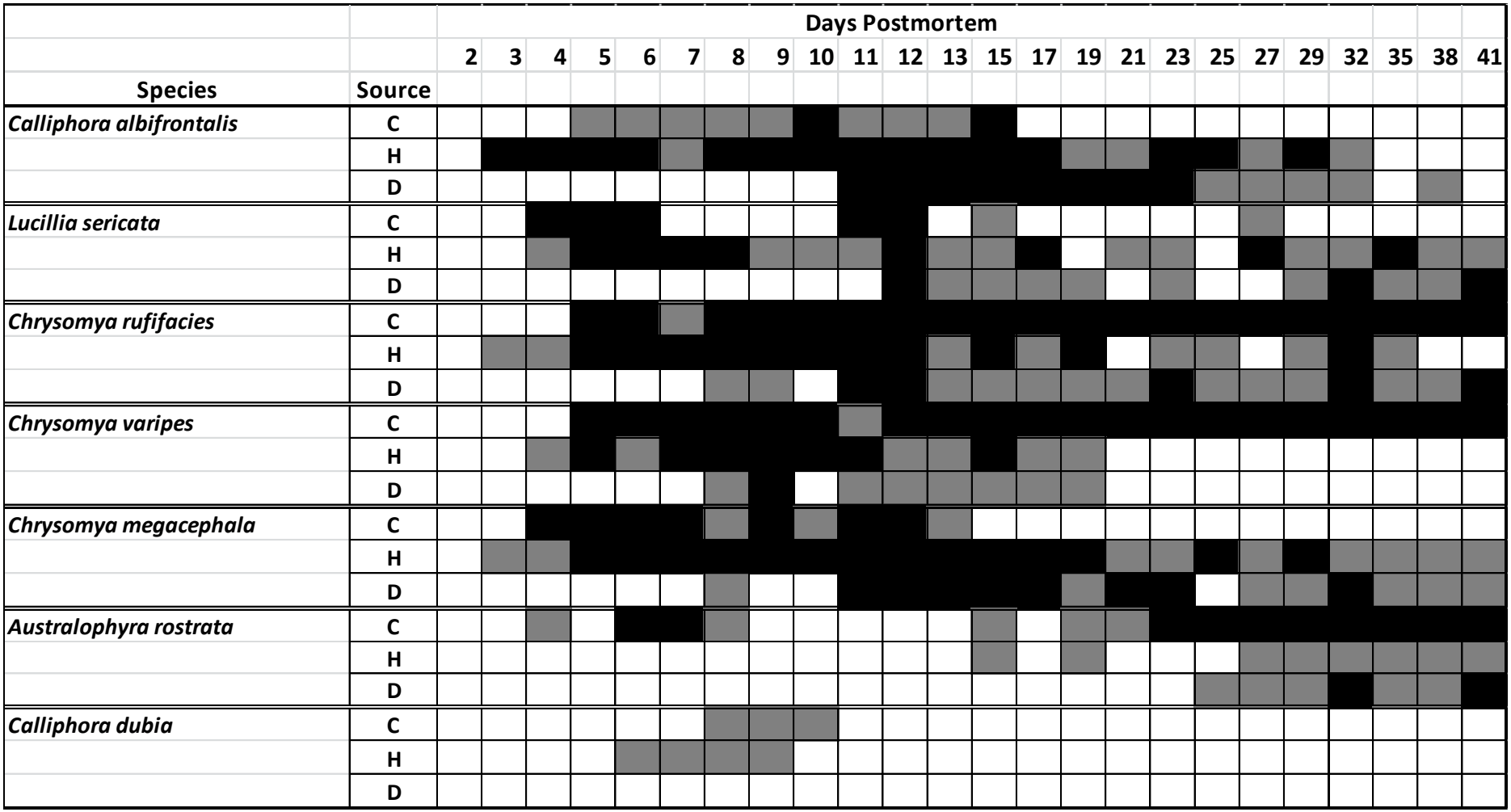


Table 14. Dipteran occurrence matrix for both treatment groups during Trial 2. Source indicates which treatment group the sample was collected including the drip zone.(C) controls; (H) hanging (D) drip zone. The black cell indicates species present on both replicates and the gray cells indicate presence on replicate only



4.3.3 Trial 3 Successional Patterns

4.3.3.1 Control Carcasses

A total of six species were identified on control carcasses throughout Trial 3. In contrast to previous trials, *C. albifrontalis*, dominated throughout the entire trial., Eggs were observed on day 2 and subsequent waves of succession were detected with eggs collected on day 6-9 and again on day 19. (Table 15; Table 16)

Calliphora dubia was collected briefly on days 5 and 6 and days 4 and 11 for each control with one control showing small collections of *C. varifrons* and *L. sericata* around day 7 -12.

Australophyra rostrata was a clear tertiary coloniser of the control carcasses with a strong presence being noted on days 15-19 for the control carcasses (Table 15; Table 16).

In contrast to all previous trials, *Ch. rufifacies* was infrequently collected off control carcasses and at a much later time point on day 25. Similarly, *Ch. varipes*, a previously observed dominant species of earlier trials, was not collected at all throughout Trial 3. Overall the abundance numbers of species observed was greatly reduced compared to earlier trials.

Table 15. Dipteran occurrence matrix, first control carcass Trial 3. Samples were collected daily and records indicate the day and stage of larval growth upon collection: (E) eggs, (1) first instar, (2) second instar, (3) third instar, (P) pupa(e).

	Days Postmortem:		2	3	4	5	6	7	8	9	10	11	12	13	15	17	19	21	23	25	27	29	31
Order	Genus and Species																						
Diptera	*Calliphora albifrontalis*	E	■	■				■	■	■							■						
		1		■		■	■		■	■	■							■		■			
		2		■	■	■	■		■	■	■	■	■		■	■	■	■					■
		3		■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
		P																					
	Calliphora dubia	E																					
		1				■																	
		2				■	■																
		3					■																
		P																					
	Australophyra rostrata	E															■						
		1								■							■	■	■	■			
		2																■		■	■		
		3																	■	■	■	■	■
		P																					

Table 16. Dipteran occurrence matrix, second control carcass Trial 3. Samples were collected daily and records indicate the day and stage of larval growth upon collection: (E) eggs, (1) first instar, (2) second instar, (3) third instar, (P) pupa(e).

	Days Postmortem:		2	3	4	5	6	7	8	9	10	11	12	13	15	17	19	21	23	25	27	29	31
Order	Genus and Specis																						
Diptera	*Calliphora albifrontalis*	E		■			■	■															
		1		■		■	■		■	■		■	■				■						
		2		■	■	■	■		■	■	■	■	■		■	■	■	■	■	■			
		3			■	■				■	■		■	■		■	■	■	■	■	■	■	■
		P																					
	Chrysomya varifrons	E																					
		1						■															
		2							■	■					■								
		3																					
		P																					
	Calliphora dubia	E																					
		1			■							■											
		2										■											
		3																					
		P																					
	Lucillia sericata	E						■															
		1								■		■											
		2								■	■	■	■										
		3																					
		P																					
	Chrysomya rufifacies	E																					
		1																		■			
		2																			■		■
		3																					
		P																					
	Australophyra rostrata	E																■					
		1																■					
		2													■	■					■	■	
		3															■						■
		P																					

4.3.3.2 Hanging carcasses

Seven species were collected in association with the hanging carcasses and drip zone, all consistent with one of the control succession patterns with the exception of *Ch. megacephala* which was noted once on day 13 on one hanging carcass (Table 17). Eggs and second instar maggots of *C. albifrontalis* were collected off both hanging carcasses on day 3 of Trial 3, indicating the presence of the species was likely there within 24 hours of the carcasses being hung out in the field (Table 17; Table 18). The blowfly, *C. albifrontalis*, was again the dominant species collected throughout this trial with representatives collected continuously over the remainder of the trial period. For both controls, *C. dubia* was detected by day 5, whilst on the hanging carcasses *C. dubia* was detected on day 8. At no time was *C. dubia* collected as a third instar or pupa off any of the replicates during this trial., Eggs of *L. sericata* were collected on day 7 from one of the control carcasses but were not detected on hanging carcasses until day 8 and 10 (Table 19).

Australaphyra rostrata was collected from both controls and found directly on one of the hanging carcasses, and in the drip zone related to the other hanging carcass. The earliest detection of *A. rostrata* was off one of the control carcasses on day 9, and the hanging carcass on day 10 as eggs and first instar maggots. It was later collected off both the control carcasses beginning on day 15 and furthermore, in the drip zone of both hanging carcasses by day 25 and 27 (Table 19).

In general, within Trial 3, insect succession was similar between treatments. The limited presence of hairy maggots (*Ch. rufifacies* and *Ch. varipes*) and dominance of *C. albifronatlais* was similar between controls and hanging carcasses (Table 19).

Table 17. Dipteran occurrence matrix, first hanging carcass Trial 3. Samples were collected daily, and records indicate the day and stage of larval growth upon collection: (E) eggs, (1) first instar, (2) second instar, (3) third instar, (P) pupa(e). If specimens were collected directly from carcass this is indicated by the (H) column. If specimens were collected from the drip zone, this is indicated in the (D) column.

	Days Postmortem:		2		3		4		5		6		7		8		9		10		11		12		13		15		17		19		21		23		25		27		29		31	
	Zones		H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D
Order	Genus and Species																																											
Dipera	*Calliphora alibifrontalis*	E			■		■		■		■				■		■		■				■																■							
		1			■		■										■		■		■		■		■		■		■		■		■	■	■									
		2			■		■						■						■		■		■		■		■		■		■		■	■	■		■				■		■	
		3									■		■		■						■		■		■		■		■		■						■	■		■	■	■	■	■
		P																																										
	Calliphora dubia	E									■																																	
		1													■																													
		2													■																													
		3																																									
		P																																									
	Lucillia sericata	E																	■																									
		1																			■																							
		2																			■																							
		3																																									
		P																																									
	Chrysomya megacephala	E																							■																			
		1																																									
		2																																									
		3																																									
		P																																									
	Chrysoma rufifacies	E																																	■			■					
		1																																		■	■						
		2																																									
		3																																						■	■	■	■	■
		P																																									
	Australophyra rostrata	E																																									
		1																																									
		2																																			■		■					
		3																																						■		■		
		P																																									

Table 18. Dipteran occurrence matrix, second hanging carcass Trial 3. Samples were collected daily, and records indicate the day and stage of larval growth upon collection: (E) eggs, (1) first instar, (2) second instar, (3) third instar, (P) pupa(e). If specimens were collected directly from carcass this is indicated by the (H) column. If specimens were collected from the drip zone, this is indicated in the (D) column.

	Days Postmortem:		2		3		4		5		6		7		8		9		10		11		12		13		15		17		19		21		23		25		27		29		31	
	Zones		H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D
Order	Genus and Species																																										
Dipera	*Calliphora alibifrontalis*	E			■		■		■		■		■		■																													
		1			■										■		■				■				■		■		■				■											
		2			■																		■		■		■		■		■				■			■		■				
		3															■		■		■		■		■		■				■		■		■		■	■	■	■	■	■	■	■
		P																																									
	Calliphora varifrons	E																																									
		1															■																											
		2																	■																									
		3																			■																							
		P																																									
	Calliphora dubia	E													■																													
		1													■																													
		2															■						■																					
		3																																									
		P																																									
	Lucillia sericata	E																																									
		1													■																													
		2																	■						■																			
		3																					■		■						■	■									■			
		P																													■												
	Chrysoma rufifacies	E																																									
		1																																									
		2																																					■		■		■	■
		3																																							■	■	■	■
		P																																										
	Chrysoma varipes	E																																									
		L																																									
		P																																									
	Australophyra rostrata	E																	■																									
		1																	■										■															
		2																													■				■				■				
		3																													■				■				■	■		■		■
		P																																										

Table 19. Dipteran occurrence matrix for both treatment groups during Trial 3. Source indicates which treatment group the sample was collected including the drip zone.(C) controls; (H) hanging (D) drip zone. The black cell indicates species present on both replicates and the gray cells indicate presence on replicate only

		Days Postmortem																				
		2	3	4	5	6	7	8	9	10	11	12	13	15	17	19	21	23	25	27	29	31
Species	Source																					
Calliphora albifrontalis	C																					
	H																					
	D																					
Calliphora dubia	C																					
	H																					
	D																					
Calliphora varifrons	C																					
	H																					
	D																					
Lucillia sericata	C																					
	H																					
	D																					
Australophyra rostrata	C																					
	H																					
	D																					
Chrysomya megacephala	C																					
	H																					
	D																					

4.4 **Comparison of Insect Succession between Trials 1, 2 and 3**

Overall, insect assemblages were similar between the control and hanging carcasses within the trials, although oviposition on the hanging carcasses was sometimes delayed. The composition of assemblages, arrival times and species numbers varied significantly between the three seasons (Trial 1: early autumn, Trial 2: late autumn and Trial 3: spring). Insect oviposition sites did not vary considerably, with the natural orifices in the head and groin region for both the control and hanging carcasses being colonised first.

Trial 1 was the warmest month with temperatures during the trial regularly reaching above 30°C during the day. Species richness was greatest throughout Trial 1 with the species *Lucilia cuprina, Piophila casei,* and *Sarcophagidae sp.* only colonising carcasses during this trial (Table 4). Larval abundance and range of life stages for a species at any one time was also greatest for carcasses exposed in Trial 1 compared to carcasses in the other trials. For instance, on a given day the same species would be collected as first, second and third instar maggots suggesting multiple successional waves of oviposition.

In regard to the comparatively cooler Trial 2 and Trial 3, while carcasses experienced similar ambient temperatures, insect succession and dominant taxa varied between the seasons. There was a delay in oviposition on the hanging carcasses for several species identified in Trial 1, and Trial 3. During Trial 1, the assemblage of species was similar on the control and hanging carcasses, and the pattern at which species came in succession of each other was also similar, but oviposition was significantly delayed for the secondary coloniser species on the hanging carcasses by 3 days (Table 7, Table 8). A delay of oviposition on the hanging carcasses was also noted in Trial 3, where results indicated the primary coloniser species for both the controls and hanging carcasses (*C. albifrontalis*) was delayed by 1 day on the hanging carcasses.

Either *C. albifrontalis,* or *C. dubia* were identified as primary coloniser species for each trial on both the hanging and control carcasses, with the exception of Trial 2 control carcasses that identified *L. sericata* and *Ch. megacephla* as the primary

colonisers. These species remained dominant secondary coloniser species for most trials.

Australophyra rostrata was identified as a clear tertiary coloniser species throughout trial period. Although, detection of this species was also made very early on in both control carcases during the Trial 2 (Table 10, Table 11)

Discussion

The main objective of this study was to provide baseline reference data on the insect succession and the rate of decomposition for hanging carcasses in Western Australia. Decomposition processes and insect colonisation of hanging pig carcasses were compared with carcasses decomposed on the soil surface. Seasonal conditions, particularly temperature, are known to influence both the rate of decomposition and the carrion community associated with carcasses throughout the stages of decomposition (Mann et at., 1990; Goff, 2010; Byrd & Castner, 2010). As such, this work considered the effect of temperature and season by conducting trials under three time periods. Data compiled here encompasses early autumn (Trial 1: 19th March - 18th April 2013), late autumn (Trial 2: 30th April - 12th of June 2013) and early spring (Trial 3: 13th September - 14th October 2013). Consequently, data generated here are most appropriately applied to death scenarios where similar climatic and environmental conditions were experienced in Perth, Western Australia (Mann et at., 1990; Campobasso & DiVella, 2001; Lopes de Carvalho & Linhares, 2001).

Focus was placed on the succession of Diptera, as representatives of this order were considered the primary indicator species for mPMI. Differences were evident in the rate of decomposition across trials and within each trial between hanged and control carcasses. The associated pattern of insect succession onto carcasses differed considerably across trials.

5.1 **Decomposition**

Within treatments (hanging and controls), decomposition rates were generally consistent within trials, although there was a slight difference in the onset of decay between the hanging carcass replicates within Trial 1 and Trial 2. This is likely a consequence of difference in maggot mass size between treatment groups.

The rate of decomposition varied between the hanging and control carcasses during each trial, and considerable differences in decomposition rates were observed when comparing across trials. Decomposition of hanging carcasses differed compared with

control carcasses in respect to overall retention of carcass biomass throughout the stages of decomposition. This observation was consistent throughout each of the trials, although the observed retention of flesh increased for all treatment groups during the cooler trial periods. The retention of flesh and subsequent mummification of the hanging carcasses can be attributed to several factors including increased surface exposure to the elements (wind, sun) and a lower level of maggot massing activity observed on hanging carcasses compared to control carcasses, and exclusion of soil dwelling microbes which can enhance the decomposition process (Bornemissza, 1956; Bereuter et at., 1997).

Mummification is often reported as the product of desiccation, and a process that occurs when moisture is rapidly removed from the soft tissues due to climatological conditions. Mummification is often associated with hot and dry climates due to the rapid loss of moisture from a carcass, although this can also occur in very cold climates (Sledzik & Micozzi, 1997; Galloway, 1997). Data reported here indicate that while climatological conditions cause mummification the physical state of the body during decomposition can enhance the conditions that lead to mummification. The hanging carcasses experienced the rapid onset of mummification in the ears and lower back of the hanging carcasses whilst exposed to varying temperatures, and rainfall. Due to increased surface area exposed to airflow while hanging, lack of soil dwelling microbes and moisture retention from contact with the soil surface, the hanging carcasses were observed drying out in areas on the face, scalp, ears and lower back much faster than the control carcasses. Disintegration of the epidermis during mummification leads to a loss of proteins and a predominance of triacylglycerols as a result of lipid degradation (Bereuter et at., 1997). Dipteran maggots will not feed on the triacylglycerols formed in the process of mummification (Bereuter et at., 1997) which may account for the reduced feeding activity of maggots observed on hanging carcasses (also supported by prolonged biomass retention) compared to control carcasses.

Gennard (2012) suggested that mummification of a hanging carcass will cause less feeding by maggots and therefore decomposition will be slowed down in comparison to control carcasses. However, in this study lower predation of maggots increased the onset of the post decay, as maggot activity ceased quicker on a majority of the hanging carcasses then the control carcasses and less predation of skin occurred.

Maggot massing is active during active decay, and once these masses cease it acts as the onset of post decay. Although the skeletal stage was not reached within the duration of the trials, due to the increased biomass retention in the post decay phase of decomposition for the hanging carcasses, it is likely that a hanging carcass would take much longer to reach a skeletal stage in comparison to carcasses laying on the soil surface. These findings were supported by Goff and Lord (1994) when it was noted that hanging altered the drying pattern of the body and excluded soil-dwelling taxa, and suggested that the exclusion of Dermestidae beetles from the hanging carcass only further delayed the complete breakdown of the body to skeletal stage.

While retention of skin is primarily attributed to mummification (Bereuter et at., 1997), reduced abdominal maggot masses present in the hanging carcasses also acted as an influence to the increased amount of skin retained on the hanging carcasses into post decay. Both these processes affected one another, with the onset of the initial stages of mummification in the hanging carcasses occurring quickly making it harder for maggots to penetrate the top layers of skin due to drying from the surrounding air around the abdomen. This is an area of high biomass removal in carcasses laying on the soil surface as masses are able to form large interconnecting groups which actively remove much of the tissue during the decomposition process with the right conditions (Trial 1 & 2; as adipocere tissue was formed in Trial 3). Masses also commonly form along the soil to carcass interface where soil microbes, and moisture weaken the tight interlocking surface epithelial tissues making it much easier for feeding to begin in areas of tiny skin fistures and skin cracking. Lack of maggot masses forming in this area on hanging carcasses during all trials, is the effect of both these processes occurring in conjunction with one another. The rapid drying of the outer layers of the skin which begins immediately in the hanging carcasses makes it hard for maggots to break through the outer epithelial layers and establish a place for feeding. Whilst the elevation of the body also reduces the number of maggots able to grasp onto the abdominal area as they are affected by gravity and were often observed falling off if they travelled towards the abdomen of the hanging carcasses.

Maggot masses are known to increase the rate of decomposition due to accelerated temperatures and increased feeding activity (Rodriguez & Bass, 1982; Kočárek, 2003; Cockle & Bell, 2015). Lack of maggot masses forming in the abdominal region

for the hanging carcasses is consistent with findings of other hanging trials (Goff & Lord, 1994; Shalaby et at., 2000, Chin et at., 2010), and this in conjunction with drying of the flesh likely cause the observed retention of biomass in the hanging carcasses compared with the control carcasses. During Trial 1, (the only trial where skin splitting occurred in the hanging carcasses) dead third instar maggots were observed mixed in with the internal flesh and decomposing matter that fell from the abdomen during the onset of active decay. In all trials small masses were observed trying to establish under pockets of epithelial tissue during the bloat phase, although the first instar maggots appeared to be unable to penetrate through the stretched taught skin of the abdominal areas in the hanging carcasses. It is likely at this stage the skin had already behun to dry out and maggots could not chew through the outer dried layers, and before feeding activity could establish, the maggots dispersed or fell from the body. Because the surface area for supporting a large mass is reduced in the hanging carcasses, and the effects of gravity, maggots were frequently observed falling from the mouth and groin area and into the soil below. As these areas were the primary sites for maggot massing in the hanging carcasses, this is likely to have significantly reduced the productivity and removal of flesh in these areas, which attributed to increased mummification and observed biomass retention in the carcasses. These results are consistent with Shalaby et at., (2000), who suggested that lack of maggot massing on the hanging carcasses can be attributed to the increased biomass retention seen during later stages of decomposition.

Environmental conditions and temperature are widely known to have an effect on the rate of decomposition, and as expected our results indicated that as well as reduced maggot massing on the hanging carcasses, time of year which intern changed the ambient temperature played a significant role in the way the hanging bodies decomposed (Reed, 1958; Tullis & Goff, 1987; Dillon & Anderson, 1995; Matuszewski et at., 2010a). Temperature directly affects bacterial growth (optimum temperature for growth for bacteria ranges between 15 – 37 °C) (Galloway, 1997). During Trial 1, the warmer part of early August, ambient temperatures ranged within this spectrum, which would have enabled optimum bacterial growth in the hanging carcasses. The general pattern of decomposition observed in the hanging carcasses during Trial 1 was in line with the most widely accepted indicators of decomposition stages described from carcasses laying laterally on the soil surface (Goff, 2010). A

commonly accepted entry point to active decay stage of decomposition in carcasses laying laterally on the soil surface is when the abdominal region splits due to the built up pressure of gasses emitted during bloat, and maggot mass feeding (Goff, 2010). Chin et at., (2010) reported that by day six, bloat had subsided and abdominal splitting was observed in a hanging carcass. This was consistent with results observed in Trial 1, with the hanging carcasses abdomens splitting on days 5 and 7 within 1 days of the control carcasses splitting. It appears that although the hanging carcasses supported a lower number of maggot masses than the control carcasses, bacteria present in the gut of the hanging carcasses, working under optimum temperature conditions, is enough to cause a similar progression of decomposition commonly observed in carcasses laying laterally on the soil surface during warmer temperatures.

Abdominal splitting in the hanging carcass did not occur during trial 2 or trial 3, where temperatures were vastly cooler then experienced in trial 1. During these trials oviposition on the hanging carcases was concentrated to the natural orifices in the head and groin regions. Low temperatures, lack of abdominal splitting and concentrated areas of maggot massing, saw the concurrent presence of active decay of the head and bloat in the trunk and abdomen for the hanging carcasses. Further in the decomposition process, post decay was observed in the head, and decay was in the abdominal area. This process was far more pronounced during trial 3, and also observed in the control carcasses during Trial 3 which indicates that not just temperature, but season also plays a significant role in the process of decomposition for the hanging carcasses.

During Trial 2, there were marked differences in the observed decay rates of the hanging carcasses compared with the control carcasses. Concurrent processes of decomposition were not observed in the control carcasses during trial 2. Carcasses laying laterally on the soil surface during this time of the year still experience skin splitting throughout the abdominal cavity and progress through a natural temporal order of decomposition described in previous trials in the same geographic location (Voss et at., 2009; Voss et at., 2011). Due to the lack of abdominal splitting in the hanging carcasses, it is important to note that the hanging carcasses may appear to be a lot less aged then they actually are for this time of year. As the process of decomposition in the hanging carcasses were concentrated in the head and mouth

region during this trial, a succession of insects were observed in this area in cohesion with stages of decomposition. It is recommended that sampling of insects focuses on this site if mPMI estimates are required.

During Trial 3, there were above average, lengthy periods of rainfall. Many studies suggest that bacterial action in an excessively wet environment which causes anaerobic processes, contributes to the formation of adipocere tissue in decomposing carcasses (Yan, 2001). Adipocere results from the degradation of lipids in soft tissue and yields a soft white substance comprised predominantly of fatty acids (Fiedler & Graw, 2003). Adipocere tissue is a relatively stable product which doesn't readily decompose and maggots will not feed on it (Yan, 2001; Fieldler & Graw, 2003). Aipocere tissue was formed in the fatty abdominal areas of the control and hanging carcass during trial 3, enhancing the appearance of concurrent decomposition processes. These results are consistent with seasonal data reported by Matuszewski et at., (2010a). Again, because maggot massing was concentrated in the head and mouth region, it is recommended maggot collection is focused on this area if mPMI estimates are required under similar conditions.

In one of the only two other reported trials on hanging, Shalaby et at., (2000), suggested that the lack of maggot masses on the carcasses delayed the onset of decomposition stages. Our results do not support this finding with a delay in the onset of each decomposition stage only noted under the warmer climatic conditions of Trial 1. Shalaby et at., (2000) conducted one trial during warmer months and thus the results do not accurately reflect processes which occur in different seasons, especially cooler climates. As the hanging carcasses supported much smaller masses than control carcasses, the period of maggot activity in the hanging carcass was much shorter. This time frame of maggot activity directly correlated with the assessment of decomposition stages. So whilst Trial 2 and 3 experienced concurrent presence of decomposition processes, once maggot activity was very active in the mouth and groin region, decay was pronounced. As when maggot activity ceased in these areas, post decay was pronounced.

5.2 **Insect succession**

Previous research in Western Australia has already identified differences in insect succession patterns between different seasons and geographic regions for surface carcasses (Voss et al., 2009; Voss et al., 2011). However, it is currently unknown whether patterns of insect succession differ when a carcass is placed in a hanging position. The mechanisms which blow flies use to find a suitable breeding resource involves a series of steps including activation of interest, orientation and site assessment, ending with actual oviposition (Ashworth & Wall, 1994). Research has indicated that visual and tactile cues also play a role in this process (Goff, 2010). Therefore understanding how dipteran species respond to a hanging carcass is vital to making correct mPMI estimates as elevation of the body could influence this significantly. Goff & Lord, (1994) suggested that hanging altered insect colonisation because soil dwelling taxa were excluded, and this changed the drying pattern of the body which in turn changed the succession of flies onto the carcass. Our results indicate that the rate and way the body decomposes in a hanging carcass varies from a carcass laying laterally on the soil surface, therefore understanding if this has an effect on the succession of insect onto a hanging body, and understanding the yearly timing of this succession, is critical to making correct mPMI estimates.

Differences in insect fauna assemblages were apparent across the three trials, with a lower number of abundances for species during trial 3, the Spring trial., These results are consistent with the low number of species observed during the cooler months as reported in Voss et at., (2009) and comparative seasonal studies done internationally (Le Blanc & Strongman, 2002; Matuszewski et at., 2010b). The data generated in this report is climatically and seasonally specific, and when applied to predictions for similar death scenario conditions, regardless of comparative temperatures as seen in Trial 2, and 3, seasonal differences (difference in species pesent in late Autumn compared with Spring) in dipteran assemblages needs to be taken into account when making mPMI estimates from succession data. Applying succession data collected from similar climatic conditions but different seasons could amount to incorrect estimations of mPMI.

As expected, the assemblage of species was most abundant during Trial 1. This trial was conducted during March – April (early Autumn) and was the warmest trial of the

experiment. During this trial, there were light, intermitted patches of rain which kept moisture in the carcasses and provided an optimal environment for maggot feeding and development. A well-defined temporal pattern of insect succession was recorded from both treatment groups (controls and hanging carcasses) during trial 1. Results indicated the hanging carcasses had a higher number of taxa which dominated the succession as opposed to the control carcasses which were mostly dominated by three key species. *C. dubia* was the primary coloniser species for all carcasses and appeared by day 2 as first instar maggots on all carcases. However, detection of *C. dubia* ceased with the arrival of *C. rufifacies* and *C. varipes* on the control carcasses by day five of the trial. Both *C. rufifacies* and *C. varipes* remained the dominant secondary coloniser species on the control carcass. The arrival of *C. rufifacies* and *C. varipes* was delayed on the hanging carcasses, and because both species are known to predate smooth maggots (McLeod & Anderson, 1992; Byrd & Butler, 1997: Swindle, 2014) this is most likely linked with the higher number of species present on the hanging carcasses. The delayed arrival of the hairy maggots onto the hanging carcasses did not supress the earlier smooth maggots from establishing, and greater presence of smooth maggots were seen throughout hanging carcass decomposition. LeBlanc (2008) has found that the change in composition and concentration of volatiles change in sync with the stages of decomposition. Therefore delayed arrival of *Ch. rufifacies* and *Ch. varipes* may have been due to the release of different volatile chemicals coming from the hanging carcasses because the hanging carcasses pattern of decomposition was different from the control carcasses (retention of flesh, onset of mummification). This could have potentially varied the release of decomposition volatiles and altered the pattern of succession onto the carcasses. However, further research would need to be undertaken to establish this theory.

The black carrion fly, A*. rostrata* was a clear tertiary coloniser species on both the control carcasses during trial 1, however this species was absent from being directly collected off the hanging carcasses. Detection of *A. rostrata* occurred in the drip zone of the hanging carcasses and this is likely due to its affiliation with soil, where specimens are most often found in the fluid soaked soil beneath carcasses (Byrd & Castner, 2010).

Trial 2 was conducted towards the end of autumn, and continued slightly into the winter season. The beginning of the trial experienced warmer days, but as the trial continued it got increasingly cooler as expected. Changing seasons and decreasing temperatures is the likely reason both treatment groups saw the early arrival of many species normally detected as secondary or tertiary species. The early arrival of several species was clustered in the early days of the trial for all carcasses, however, there were differences in the control and hanging carcass succession patterns. *L. sericata* and *C. megacephla* were detected as the primary coloniser species on the control carcasses, whilst *C. albifrontalis* was the primary coloniser for the hanging carcasses. *C. albifrontalis* was detected on the control carcasses soon after primary oviposition, however did not remain as active on the control carcasses as the hanging carcasses and this identifies *C. albifrontalis* as a key forensic indicator for hanging carcasses during late autumn. *Ch. rufifacies* and *Ch. varipes* arrived slightly earlier on the hanging carcasses compared with the control carcasses, however they were less dominant on the hanging carcasses and this saw a persistence in the presence of the smooth maggots on the hanging carcasses. This was not the case for the control carcasses, as *C. albifrontalis, L. sericata* and *C. megacephla* were seen to dwindle after the arrival of *Ch. rufifacies* and *Ch. varipes.* A well-known action of the hairy maggots is to predate the primary coloniser species as they are in direct competition for the food source, a process which has been reported several times in forensic literature (McLeod & Anderson, 1992*;* Swindle et al., 2014). Similarly with Trial 1, these results indicate that the hanging carcasses do not support large masses of *Ch. rufifacies* and *Ch. varipes* as they cannot form large dominanting masses on the hanging carcass, and this effects the persistence of smooth maggots on the hanging carcasses after the arrival of these two hairy maggots species (Swindle et at., 2014). The very early arrival of *Ch. rufifacies* and *Ch. varipes* (normally secondary coloniser species) is likely due to the considerable change in season experienced during this trial which saw *Ch. rufifacies* and *Ch. varipes* utilising the last of the warmer months before winter dormancy set it, and dominant *C. albifrontalis* activity was supported by increased numbers due to the onset of cooler weather.

A. rostrata was detected on the control carcasses early in the decomposition process during Trial 2, and unlike Trial 1, this species was detected directly on the hanging

carcasses, as well as in the drip zone, although it was only detected as a late stage tertiary coloniser species on the hanging carcasses. This suggests that *A. rostrata* will favour food resources which are found in direct contact with the soil surface (large drip zone of Trial 1), however, where this is limited as in Trial 2, oviposition will occur directly on the hanging carcass.

Trial 3 began in early spring, and rainfall was significantly above average during this trial period. The absence of *Ch. rufifacies* and *Ch. varipes* from the beginning to mid half of this trial meant both the control and hanging carcasses were dominated by the arrival of *C. albifrontalis* which remained active on both treatment groups for the duration of the trial. *Chrysomya rufifacies* and *Ch. varipes* are known as species which prefer warmer weather. They remain dormant throughout the coldest months and only emerge when the weather becomes warmer (Morris & Dadour, 2005). Sporadic, continued oviposition of *C. albifrontalis* was detected on the hanging and control carcasses throughout the trial. No difference in the persistence of smooth maggots was detected, because hairy maggots never took hold of the controls as seen in the previous two trials likely due to dormancy delaying their arrival to the carcass. Towards the end of the trial as the weather gradually began to warm up, *Ch. rufifacies* was detected as a tertiary coloniser species, on both treatment groups. First detection of *Ch. rufifacies* was made on day 23 for the hanging carcasses, and day 25 for the control carcasses, however large masses were not established, and at this stage in the trial the carcasses were predominantly composed of adipocere tissue which maggots will not eat. These results are supported by similar seasonal trials done in the same geographical location (Voss et al., 2011), and are consistent with the seasonal laying behaviour of *C. albifrontalis* that are more predominant in cooler months after winter.

The results presented here are consistent with Voss et al., (2009) findings on species abundance and succession rates conducted in two locations in Western Australia during two different seasons. Voss et al., (2009), noted the inclusion of several species during the summer months which were not present during the winter trials and that decomposition rate was slowed down by the cooler climate present during the winter season. Our results were also consistent with international seasonal patterns of decomposition and insect succession research (Centeno et at., 2002; Wang et at., 2008) which showed that during the warmer months, a greater

number of insects visited the bodies and decomposition rate was significantly faster than that of the winter trial periods. Within trials, differences in the insect succession and assemblage of hanging and surface carcasses were driven by the observed lower abundance of *Ch. rufifacies* and *Ch. varipes* on hanging carcasses. Suspended carcasses appear to support lower numbers of hairy maggots which corresponds with the prolonged presence/dominance of smooth maggots on hanging carcasses compared to surface carcasses.

Further to the rates of succession on the hanging carcasses, Shalaby et at., (2000) suggested, that the drip zone site below the hanging carcasses re-created and environment closer to that of carcasses in contact with the soil surface. Therefore, insects collected from this area may more accurately reflect the total period of insect activity than those collected from the hanging remains. Our results completely discount this as it was established that throughout the different ambient temperature periods, even during Trial 2 and Trial 3 where ambient mean temperatures were similar, the contrasting environmental conditions for each trial significantly influenced the onset of the drip zone. As well as this, at no time were eggs collected directly from the drip zone area. Whilst this doesn't completely discount primary and secondary indicator species having laid their eggs directly onto the drip zone site, there is no reliable indicator of the original source of maggots observed in the drip zone. Maggots were observed actively falling from the carcass into the drip zone area and, as the sizes of the maggots collected from the drip zone and carcass on the same day are comparatively similar, it is more likely that the majority of maggots found in the drip zone originated from oviposition on the carcass itself.

One exception needs to be noted and that is of A*. rostrata,* in Trial 1. During this trial, skin splitting occurred and a large mass collected in the drip zone area below the carcass. *A. rostrata* was collected exclusively from this area, beginning on day 8 as first instar maggots, and continued being collected for the duration of the trial only in the drip zone. When a majority of maggot activity had ceased on the hanging carcass, *A. rostrata* were observed to remain active in the drip zone area during Trial 1, as moisture was retained in the soil and supported continued growth. It is likely that after all maggot activity had ceased on the carcass *A. rostrata* remained active for a period of time in the drip zone, although our trials did not permit enough time to record this. A. *rostrata* are known to prefer development in fluid soaked soil, and are

often found underneath the remains of a body (Byrd & Castner, 2010). During Trial 2 and Trial 3, the abdomens didn't split during decomposition, and therefore there was very little physical matter in the soil below the carcass. *A. rostrata* was detected on the hanging carcasses midway through the trial period, before being detected during the end of both trials as 2nd and 3rd instar maggots in the drip zone. It is likely that due to the lack of contents in the soil below the female *A. rostrata* oviposited on the hanging carcass, although this species appears to prefer to oviposit in the drip zone and into soil, if decomposing matter is present.

Studies have also highlighted the effects of starvation on maggot growth, and Singh & Bala (2009) reported that *C. megacephala* and *Ch. rufifacies* needed to have consumed food for a period of 35 hours in 28 °C in order to be capable of reaching post feeding stage of maturation and subsequent pupation. As maggots of both species were observed falling from the carcass as first and second stage instar sizes it is likely their maturation was distorted in the nutrient deprived drip zone areas during Trial 2 and 3. This would need to be taken into consideration if making mPMI estimates from maggots collected in this area.

The variability of the formation of the drip zone, and the different circumstances surrounding the arrival of species to the area all leads to the conclusion that this site is not appropriate for collection of maggots for mPMI estimates.

5.3 **Conclusion**

Forensic entomology offers one of the most accurate means of estimating mPMI where time of death and deposition of the body coincide (Catts & Goff, 1992; Morris & Dadour, 2005; Byrd & Castner, 2010; Goff, 2010). However, the rate of decomposition and associated insect succession pattern onto a decomposing carcass can be affected by a range of different biotic and abiotic factors such as, environmental conditions (Ames & Turner 2003), seasonal variations (Matuszeweski 2010a), geographic location (Cockle, 2013), physical state of the carcass (Avila & Goff, 1998), as well as body size and condition (Shalaby et at., 2000). These factors can drastically change the time frame of species arrival and departure on a decomposing body. Therefore, the need for geographically specific, baseline

reference data encompasing a range of decomposition scenarios is of utmost importance for forensic investigators to compare findings with to promote accurate mPMI estimates.

As most succession studies are conducted using carcasses exposed on the soil surface, this study attempted to address some the current deficit of knowledge relating to the decomposition of a hanging carcass and its affect, if any, on the succession of insects onto the carcass. Knowledge of how a hanging carcass decomposes, and its influence on insect succession can provide extended more precise reference data for making mPMI and reduce the need for interpretation of succession time frames collected from carcasses on the soil surface.

Results suggest differences in the rate of decomposition and insect succession onto hanging carcass's throughout the year and minimal differences compared to that of surface carcasses. During climatic conditions of Trial 1 and Trial 3, the hanging carcasses decomposed in a similar pattern to carcasses laying on the soil surface, however, during the transition from autumn to winter (Trial 2) the hanging carcasses rate of decomposition was different to the control carcasses. Rates of decomposition were directly associated with the patterns of insect succession and during the warmer months, where drying of the epithelial tissues and the onset of mummification was observed to occur faster in the hanging carcasses. Variation in patterns of insect succession between the surface and hanging carcasses were also apparent during Trial 2. However, Trial 3 successional patterns were consistent with the control carcasses. This appears to be a reflection of the reduced seasonal presence of *Ch. rufifacies* and *Ch. varipes* on both hanging and control carcasses. In Trial 1 and 2, the greater colonisation dominace of surface carcasses by *Ch. rufifacies* and *Ch. varipes* compared to hanging carcasses was linked to successional differneces in the persistence of smooth maggot taxa between treatments.

Thus, while season impacted on the dominant species observed to colonise hanging and surface carcasses, the key differences in insect succession between hanging and surface carcasses were directly linked to the arrival and dominance of *Ch. rufifacies* and *Ch. varipes*. Even during periods where *Ch. rufifacies* and *Ch. varipes* are abundant, suspended carcasses appear to support lower numbers of hairy

maggots. This corresponds with the prolonged presence/dominance of smooth maggots on hanging carcasses compared to surface carcasses.

This study has highlighted the variability in rates of decomposition, key processes which describe the onset of different decompositional stages and the differences in insect succession onto hanging carcasses at varying times of the year. Therefore, when encountering a hanging carcass to obtain maximum accuracy in mPMI estimation, it would be recommended to use the data associated with this work.

In conclusion, hanging carcasses rate of decomposition compared with carcasses laying on the soil surface varied in different climatic conditions and this had an effect on the dipteran succession and assemblage of species onto the carcass at different times of the year.

5.4 **Limitations**

Due to the prohibitive cost of >40 kg pig carcasses and suitable steel cages for hanging the carcasses only two replicates per treatment were used in each of three trials. Instead, the trial was repeated throughout the year rather than at the same time period to provide replication. Ideally, where funding is available, a study consisting of ten or more replicates could be performed in the same time period for statistical analysis of results. As the results currently stand, no statistical analysis was performed because two replicates for each month limited the sample size. Repeating the experiments in the same month over two years would have also strengthened the results obtained, because as they stand currently there is no further experimental evidence supporting the findings of each month. Including a second trial during the same period in a different year would also resolve issues surrounding the spring trial which saw an unusual high amount of rain fall for that period of the year. Repeating this experiment during the same time period in a different year however, would shed more light on the results and if they are a result of the time of year (seasonality) or weather conditions present for this time of year or both. Currently as the results stand, they would only be able to be compared to similar weather conditions for this period of time as well as the season as these weather conditions were not the norm for this time period in Perth, Western Australia.

Whilst within trial replication was limited, replication under different temperature conditions provides a starting point to develop the data for referral throughout the year. The differences reported in the results also indicate that succession patterns are very temperature and seasonally driven, which highlight the importance of matching the data used to the specifics of a death scene.

Typically, succession data collected from one season would not be relevant for a different season for a given geographic location. Therefore, many forensic entomology guides recommend performing trials over varying times of the year to reduce the chance of introducing error into mPMI estimates when applying succession data (Payne, 1965). The initial experimental plan for this study was designed to begin in late January, the end of summer for Perth, Western Australia which would have included a summer trial, however delays in the onset of field work pushed back the starting time. Including a summer trial would have put this experiment in line with the methods of other published experiments which have considered seasonal differences and included three different seasonal changes (Matuszeweski et at., 2010a; Matuszeweski et at., 2010b). A winter trial was precluded from the study, although, similarly with the summer trial, including a winter trial would only increase the knowledge of baseline reference data on hanging carcasses for Perth, Western Australia.

5.5 **Future Research**

Our study revealed that the seasonal transition from late autumn to winter had an effect on the decomposition rates of the hanging carcasses in comparison to the control carcasses, and results obtained during this trial highlight the inconsistency of applying results obtained from carcasses on the soil surface to hanging carcass decomposition. The varied rates in decomposition had a direct result on the laying behaviour and succession of species onto the hanging carcasses, and resulted in Trial 2 also having the most varied rate of insect succession between the treatment groups. As patterns of insect succession can provide valuable clues to an investigation, the basis of mPMI estimates rests on the consistency of colonisation patterns in that given geographic area. Several forensic entomology manuals suggest applying geographically specific succession data to investigations relying on mPMI estimates from insect material, however only a few succession papers stress

the importance of applying scenario specific entomology data (Voss et at., 2009). As our results indicate, the physical state of the carcass can have an effect on these succession patterns and replication of succession patterns collected during this specific seasonal times of the year would strengthen the data presented in this report, and application for real life scenarios.

For greatest accuracy, estimating mPMI from succession data requires information from local geographic areas, at different times of the year (Anderson, 2001). It is vitally important to be aware of all the factors impacting insect colonisation and decomposition rates of remains, and taking these into account when analysing a death. Including a succession trial during summer and winter, would expand the baseline data for hanging carcasses in Western Australia, and worldwide

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