Decomposition and arthropod succession on buried remains during winter and summer in central South Africa: Forensic implications and predictive analyses

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J. L. Botham

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ABSTRACT

While burial is utilised by criminals as a means of disposing of a body, knowledge of the impact it has on arthropod succession and decomposition within South Africa is currently lacking.

The study was conducted within a 24 hectare grassland field, located on the University of the Free State grounds. A total of seven pig carcasses (*Sus scrofa* Linnaeus) were utilised for each of the two seasonal trials, with six of them being buried in randomly spaced graves at depths of 60cm and one placed above-ground as a control. Each of the graves was excavated on predetermined days over each 60 day trial to minimise disturbance and evaluate the impact of differing time periods on decomposition and arthropod succession. After its initial excavation, grave one was excavated every third day until the conclusion of the trial to determine the impact of disturbance on buried remains.

Decomposition and biomass loss progressed faster on buried carcasses compared to above-ground during the winter season. A faster rate of decomposition and biomass loss was seen for the above-ground carcass versus the below-ground carcass during the summer season, mainly due to heavy rainfall causing waterlogging of the graves. Between the two seasons, a higher decomposition and biomass loss rate was recorded on the summer buried carcasses compared to those buried in winter.

Dipteran species were seen to dominate on the summer control carcass, while, during the winter trial, the coleopteran species, *Dermestes maculatus* De Geer (Dermestidae), was noted to extensively colonise and outcompete present dipteran individuals. With cold, dry climatic conditions leading to the winter control carcass undergoing a form of mummification, adult *D. maculatus* individuals were seen to congregate on the carcass and reproduce, leading to larval aggregation during the active decay stage.

During the trials, only dipteran species were found to colonise the winter buried carcasses, whereas the summer buried carcasses were colonised by two dipteran species, a predatory coleopteran species and an Acari species. Of those species colonising the winter buried carcasses, two Phoridae species were found to be the most abundant, being identified as *Megaselia scalaris* (Loew) and *Conicera tibialis* Schmitz, and colonised the buried carcasses from day 21 onwards. Later occurring dipteran species in winter included *Muscina stabulans* (Fallén) and a species of the genus *Leptocera* Olivier. Summer buried carcasses saw initial

colonisation occurring from day 21 by phorid *M. scalaris* and a coleopteran beetle species from the genus *Aleochara* Gravenhorst (Staphylinidae). Further colonisation of the summer buried carcasses was seen after 30 days, with sarcophagid pupae and the Acari species *Sancassania mycophagus* (Mégnin) being sampled from the buried carcasses.

From the analyses of data gained from the two seasonal trials, predictions were made regarding the time frames of decomposition and arthropod succession applicable to buried carcasses within central South Africa. Concurrently, alternative methodologies for burial excavations and entomological evidence collection were suggested for investigators, to take into consideration during burial crime scene investigations within central South Africa.

Keywords: Forensic entomology, Burial, Biomass loss, Methodology, Predictive analysis, Excavation and entomological collection methodologies, Soil type

UITTREKSEL

Alhoewel misdadigers dikwels van ‘n liggaam ontslae raak deur dit te begrawe, is relatief min bekend oor die impak daarvan op arthropoodsuksessie en die ontbindingsproses in Suid-Afrika..

Die studie het plaasgevind op ‘n 24-hektaar grasland op die gronde van die Universiteit van die Vrystaat. Sewe varkkarkasse (*Sus scrofta* Linnaeus) is gedurende elk van die twee seisoenale proefperiodes gebruik, waarvan ses op ‘n diepte van 60cm op ewewydige afstande van mekaar begrawe is en een bogronds gelos is as die kontrole. Begraafte karkasse is opgegrawe op spesifieke voorafbepaalde tye oor die 60 dae van die proeftydperke en sodoende is verseker dat versteuring tot ‘n minimum beperk is terwyl die impak van ‘n graf-omgewing op arthropoodsuksessie en ontbinding vasgestel is. Na die aanvanklike opgrawing van die eerste graf, was die graf vervolgens elke derde dag opgegrawe tot aan die einde van die proefperiode om die impak van versteuring te evalueer.

Ontbinding en biomassa-verlies het ‘n vinniger tempo getoon vir karkasse wat begrawe was, teenoor die bo-grondse karkas gedurende winter. ‘n Vinniger tempo van ontbinding en biomassa-verlies was vir die bo-grondse karkas getoon in vergelyking met die begraafde karkasse gedurende die somer, hoofsaaklik as gevolg van die swaar reën wat veroorsaak het dat die grafte deurdrenk geraak het. Vir die twee seisoene was ‘n vinniger tempo van ontbinding en biomassa-verlies gedurende die winter vir die begraafde karkasse ondervind.

Diptera-spesies was, getalle gewys, in die meerderheid op die kontrole-karkas tydens die somer, maar gedurende die winter het ‘n Coleoptera-spesie, *Dermestes maculatus* De Geer (Dermestidae) tot so ‘n mate oorheers dat die besetting deur Diptera-spesies minimaal was. As gevolg van die koue, droë klimaatstoestande wat daartoe aanleiding gegee het dat die winter kontrole-karkas ‘n vorm van mummifikasie ondergaan het, is volwasse *D. maculatus* indivudië na die karkas aangetrek en voortplanting het tot larwale massas tydens die aktiewe ontbindingsfase aanleiding gegee.

Gedurende die winter proeftydperk, was net Diptera-spesies op die karkasse teenwoordig wat begrawe was, teenoor die besetting deur twee Diptera-spesies, ‘n predatoriese Coleoptera-spesie, sowel as ‘n Acari-spesie gedurende die somer. Die twee Phoridae-spesies, *Megaselia scalaris* (Louw) and *Conicera tibialis* Schmitz, was die volopste op die karkasse wat begrawe was gedurende die winter en was vanaf dag 21 teenwoordig. *Muscina stabulans* (Fallén) en

‘n spesie van die genus *Leptocera* Olivier het die karkas wat begrawe was gedurende die winter op ‘n latere stadium beset. Die aanvanklike besetting van die karkasse wat begrawe was, het teen dag 21 plaasgevind gedurende die somer deur die Phoridae-spesie, *M. scalaris*, sowel as deur ‘n Coleoptera-spesie van die genus *Aleochara* Gravenhorst (Staphylinidae). Latere besetting van die begraafde somerkarkas het na dag 30 plaasgevind, waartydens sarcophagid papies sowel as die Acari-spesie, *Sancassania mycophagus* (Mégnin) versamel is.

Analise van die data van die twee proeftydperke het getoon dat voorspellings gemaak kan word om ‘n tydslyn van ontbinding en arthropoodsuksessie te bepaal vir karkasse wat in sentraal Suid-Afrika begrawe is. Alternatiewe metodes om te volg word voorgestel tydens die herwinning van liggame wat begrawe was en die insameling van entomologiese bewysstukke wat in ag geneem behoort te word tydens forensiese ondersoeke in sentraal Suid-Afrika.

Sleutelwoorde: Forensiese entomologie, Grafte, Biomassa-verlies, Metodologie, Voorspellings-analise, Karkasherwinning en entomologiese versamelings metodes, Grond tipe

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- Edgar Allan Poe

CHAPTER 1

Introduction

INTRODUCTION

1.1 Background

From its early beginnings in China during the 13th century (Sung 1981), the field of forensic entomology has grown in strength since its first acceptance as a discipline in the United States in the 1960s. Despite being a relatively young field, the application of knowledge garnered from arthropods has helped many criminal and civil investigations (Byrd & Castner 2010). The main benefit of forensic entomology is the ability to estimate a Postmortem Interval (PMI) based on insect developmental and succession patterns (Van Laerhoven & Anderson 1999). While the majority of PMI estimations are made by pathologists in regards to medical parameters, such as rigor mortis, body temperature, lividity and decomposition, such estimations become less reliable beyond 72 hours after death (Templeman-Kluit 1993; Henssge *et al*. 1995). The prolonged colonisation of insects on cadavers makes them an adequate and more reliable tool for PMI estimation during the later stages of decomposition (Kashyap & Pillai 1989).

Diptera are the most commonly utilised insects in forensic entomology due to their relatively high succession and development rates (Byrd & Castner 2010). It is widely viewed that the first insects to arrive at a body a few hours after death, and immediately oviposit, are dipteran species (Catts & Goff 1992). However, various abiotic factors, such as ambient temperature, relative humidity, heavy rainfall and strong wind speeds, impact dipteran colonisation of carcasses while in the field (George *et al.* 2013). Seasonality and diurnal cycles provide significant changes in ambient temperature and humidity, which in turn brings a change in species composition and decomposition rates (Gilbert & Bass 1967; Shean *et al*. 1993). Some factors that can directly influence PMI estimations include chemical, physical, climatic and presence of scavengers (Merritt & Benbow 2008). Despite the impact of such factors, experimentally obtained development rates of dipteran immatures at constant temperatures have yielded results in PMI estimations within the field (Arnaldos *et al*. 2005). While it is generally accepted that blow fly species do not oviposit at night (Baumgartner & Greenberg 1985; Greenberg 1985), occurrences to the contrary were discovered by Greenberg (1990) and Williams (2015). This influences investigations, and can even alter PMI estimations by up to 12 hours (Williams 2015).

Other arthropods are recorded as occurring during later stages of decomposition after the initial influx of dipteran species (Payne *et al*. 1968). While such arthropods are not considered as significant as dipteran species, they still provide insight into postmortem occurrences, particularly in cases where victims have been moved from their original location (Greenberg 1985). Coleopteran species are well known colonisers of cadavers during the later stages of decomposition, especially during advanced decay to the skeletonised stage (Smith 1986; Goyal 2012). Beetles have thus become the second most utilised insects in forensic investigations. Feeding by such species of Coleoptera, and their presence, combined with past environmental conditions help to estimate possible PMI (Nuorteva 1977). Certain coleopteran species are well known predators of dipteran immatures and can influence succession rates on a cadaver if in adequate abundance (Tabor & Fell 2005). Dermestidae species are often recorded on cadavers, particularly when the carcass is dry, as they are known pests on stored dry food products (Oke *et al*. 2014). Dermestid beetles are also known to be attracted by volatile compounds released by the cadaver during decomposition (Von Hoermann *et al.* 2011). Other such beetles utilised in forensics include species from Histeridae, Silphidae, Cleridae and Trogidae (Byrd & Castner 2010).

Burial is often used by criminals to dispose of a body in order to avoid detection. While many victims are buried in shallow graves due to geological difficulties and time constraints of the murderer, cases have occurred where the victim was buried at depths greater than 30 cm (Manhein 1997). Such occurrences impede access to a body, making it more difficult for investigators to determine PMI (Catts 1992). The effect of burial has been found to significantly reduce decomposition rates and arthropod succession while preserving evidence based on burial method (Rodriguez & Bass 1985). Environmental conditions also affect decomposition, with solar radiation and ambient temperature influencing grave surfaces (Mann *et al*. 1990). According to Johansen (1977) the thermal conductivity of soil is dependent upon the volume fraction of soil components, their conductivity and the microgeometry of the system. While soil provides insulating properties to below-ground ecosystems, such insulation is often impeded during burial, particularly in shallow graves. Differences in geological conditions, as well as altitude, bring about varying conditions under which decomposition and its rate can occur (Pope 2010 *unpublished*).

Adipocere formation is a common occurrence on decomposing buried remains within damp environments. Fatty acids, within remaining decomposing tissue, and their subsequent alkali salts form a waxy layer on cadavers known as adipocere (Moses 2012). High moisture levels

and decreased oxygen levels within graves increase the formation of adipocere due to anaerobic enzymatic hydrolysis by bacteria on body fats (Murad 2008). While cadavers containing more body fat produce more adipocere, defleshed remains have been recorded to form adipocere, albeit in smaller quantities (Moses 2012). Although colonisation by arthropods can still occur on buried carrion, such colonisation differs in regards to the species involved and succession compared to above-ground cases (Anderson 2001).

Hall (1990) stated that in order to accurately analyse arthropod activity on a cadaver, proper identification, distribution and taxonomy of forensically important arthropods is necessary for forensic investigations. Research into arthropod succession on buried cadavers is sorely lacking in South Africa with only general ecology of certain species known, particularly in the environmental sector (Villet 2011; Groen *et al*. 2015). This is due to the fact that much of the previous research into forensic entomology itself was not solely based on the context of forensics (Williams & Villet 2006). The most commonly associated arthropods with burial cases are the Phoridae, more commonly known as "Scuttle flies" (Disney 1994). These insects possess a humpbacked appearance and are only a few millimetres in size. The most common species of phorid found in burial cases is *Conicera tibialis* Schmitz, more commonly known as the "Coffin fly" (Colyer 1954). With its small stature it is able to infiltrate cemetery and clandestine graves of buried cadavers relatively easily, even a year after burial (Merritt *et al*. 2007). It is then able to oviposit on the buried cadavers and the immatures will then feed once hatched. Easton & Smith (1970) noted that phorid species are able to complete their lifecycle on a cadaver and still remain below-ground, giving rise to several generations on a single body. Cases of dipteran species other than phorids have been recorded with members from Calliphoridae and Muscidae having been sampled (Lundt 1964; Lord *et al*. 1992; Gunn & Bird 2011). In many instances, depth of burial is a major predominant factor influencing presence of arthropods. Many studies have recovered several species from carcasses buried at 30 cm, while those buried at 60 cm or deeper showed little to no arthropod activity (Rodriguez & Bass 1985; Van Laerhoven 1997 *unpublished*; Van Laerhoven & Anderson 1999).

A variety of coleopteran species have also been discovered on buried carrion with dermestid, histerid, staphylinid and carabid individuals being sampled (Simpson & Strongman 2002; Corrĉa & Mourae 2014). The majority of coleopteran species occurring on buried carrion are predacious, feeding exclusively on dipteran eggs and larvae. This interaction led Van Laerhoven & Anderson (1999) and Pastula & Merritt (2013) to suggest they are unreliable in

investigations due to their arrival being unpredictable, and are thus unreliable in the sense of succession.

Due to certain arthropods being predictable in regards to their successional behaviour on buried carrion, and the fact that delays in such succession occur with burial cases, the estimation of a Postburial Interval (PBI) rather than a PMI was suggested by Forbes & Dadour (2010). Due to the variety of factors affecting succession on buried carrion, estimation can prove problematic, as was discovered by Turner & Wiltshire (1999). Exposure of a buried carcass by scavengers led to an incorrect PMI estimation which did not support other evidence. All entomological evidence that was collected from the buried carcass only provided an estimation to the time since the carcass was exposed to the surface by scavengers and not death. Despite such an occurrence, previous research has identified species to be used in PBI estimations and have also been successfully utilised in field cases (Forbes & Dadour 2010). Seasonal arthropod activity allows for estimations of PBI over long periods of time, particularly in regards to dipteran pupae (Gilbert & Bass 1967). Potential remains in the utilisation of arthropods in determining PBI. However, in order to adequately utilise such indicators, the development of databases in regards to succession in various geological localities and seasons is necessary.

While detection of buried cadavers remains problematic, several techniques have been utilised in order to lessen search times as well as determine exact location. Vass *et al*. (2008) analysed the odours of decomposing buried human cadavers and found a total of 478 separate compounds. Of these compounds, only 30 were identified as being key markers in decomposition and detectable on the soil surface. Even though varying concentrations of these compounds were detected at certain intervals they are not unique to the chemical world and thus are common in nature (Vass *et al*. 2008). Nevertheless, the use of sniffer dogs to detect buried remains has proved fruitful, due to their heightened sense of smell, opening one avenue of detecting buried cadavers, albeit in shallow graves. Clandestine graves deeper than two feet (approx. 60 cm), however, become harder to detect, with odours being unable to easily reach the surface (Rodriguez & Bass 1985). As such other methods have been incorporated, including Sonar and ground penetrating radar (Brilis *et al*. 2000; Pringle *et al*. 2012), metal detectors (Ruffell & McKinley 2008) and excavations (Hunter & Cox 2005) to detect clandestine graves. While generally effective, such methods require specialised equipment and manpower. Use of vegetative growth has also been recommended as indicators of clandestine graves, whereby plant community structure and nutrient balance

alteration are affected by burial, leading to disturbed plant cover which is easily detectable (Caccianiga *et al*. 2012).

While certain signs are present that allow easier detection of buried remains (e.g. disturbed plant growth, depressions in soil), cases where detection was more difficult have been recorded. One such case included a clandestine burial in Costa Rica in which the deceased had been placed in a shallow grave and covered in lime and cement (Congram 2008). Presence of the cement and lime prevented soil depressions, increasing search times as well as prolonging excavation when eventually discovered. High lying ground water levels also hindered excavation efficiency and threatened loss of contextual evidence. While no sure-fire detection method of clandestine graves has yet been established, the usage of current practices can allow investigators to discover buried remains within a possible shorter time period.

With an average of between 15,609 and 17,069 murder cases reported between the period of 2010 to 2014 (S.A.P.S. 2015), South Africa is considered as having one of the highest violent crime rates in the world. This coupled with the fact that forensics is still gaining ground in this part of the world, brings to light the need for forensic entomology and the research required to advance our understanding of this discipline to better serve the country.

1.2 Project Aims and Objectives

The aims of this particular project were to record and compare the succession of arthropods in above- and below-ground cases during winter and summer, and to establish a logical biomass loss and decomposition pattern applicable to below-ground cases within central South Africa. A further aim of this study was to form predictive analyses of arthropod succession and decomposition rates that can be applied to below-ground cases within central South Africa.

The study's objectives were to determine the differences in arthropod succession in above- and below-ground carcasses between the two seasons. Additionally, it was also to record the pattern of biomass loss and decomposition of buried cadavers over the study period comparable to above-ground. These differences could then be used in establishing guidelines and predictive analyses for investigators in regards to burial cases.

It was hypothesised for the study that:

1. Arthropods will be able to colonise cadavers buried at 60 cm.
2. These arthropods will be able to colonise buried cadavers faster during the summer season.
3. A higher species abundance and diversity will occur below-ground during the summer season compared to the winter season
4. Coleoptera species will play a significant role in below-ground succession.
5. Decomposition will be slower below-ground in both seasons based on previous studies.
6. Greater biomass will be lost above-ground during both seasons

CHAPTER 2

Materials and Methods

MATERIALS AND METHODS

2.1 Study Site

Both seasonal trials were conducted in a grassland area of the Free State Province within central South Africa. An open field located on the campus grounds of the University of the Free State was utilised for the trials ($29^0$8'S; $26^0$10'E, ± 1560m above sea level) (Figure 2.1).

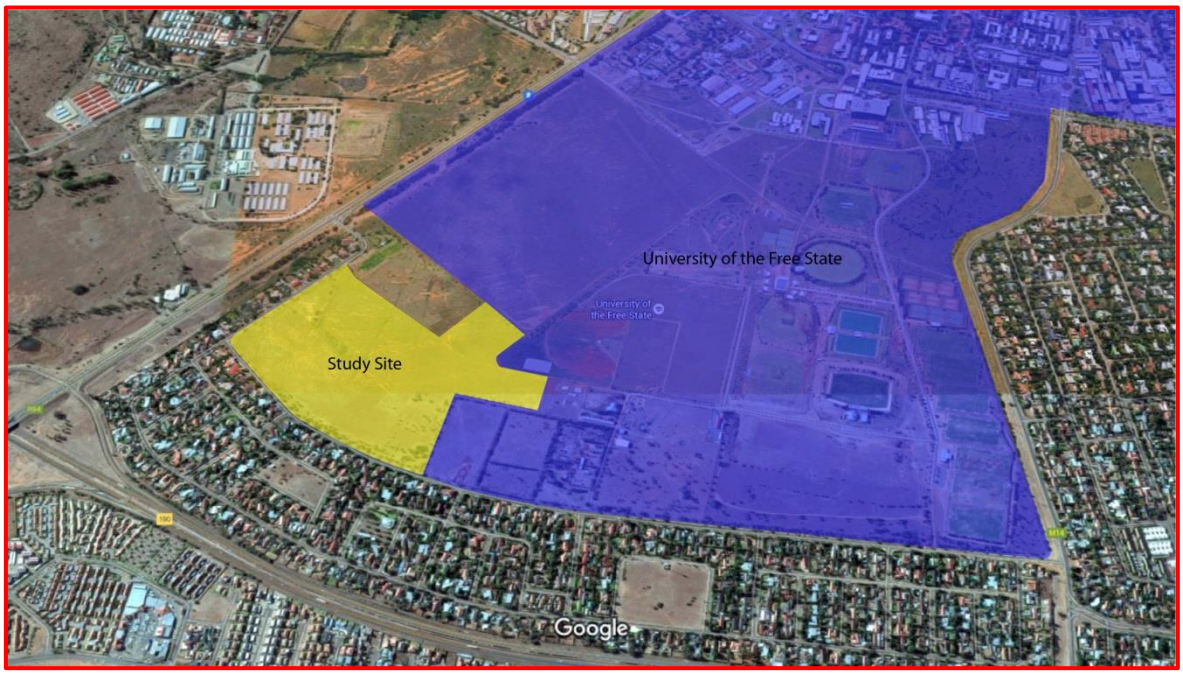

Figure 2.1: Aerial photograph of the study site (Yellow) on the University of the Free State grounds (Blue) (Image altered from Google Maps, accessed 15 September 2015).

The open field consisted of approximately 24 hectares of grassland with a few trees present. Annual ambient temperatures in the field were subjected to a very hot summer temperature range of 21^0C to 44^0C and a winter temperature range of 13°C to 28°C. Expected annual precipitation for the area ranged from 350-400mm, concentrated over the summer months (MacKellar *et al*. 2014).

A number of grass and tree species were present in the locality with *Themeda triandra* Forsk dominating the grass species. The majority of trees consisted of the species *Rhus rehmanniana* Engl. and *Vachellia karroo* (Hayne) (formerly *Acacia karroo* (Banfi &

Galasso 2008)). During the two seasons, plant growth varied significantly within the field (Figure 2.2).

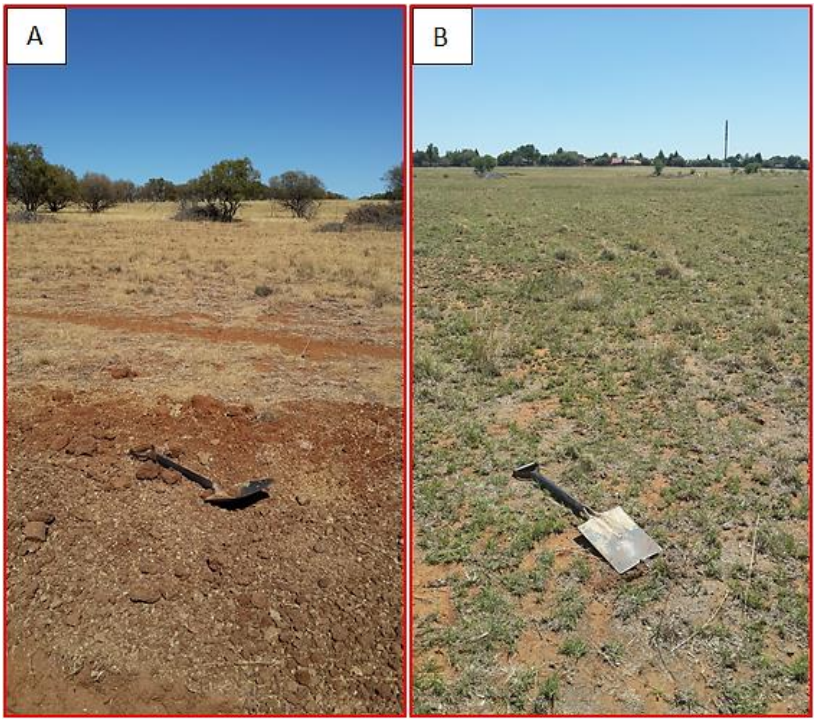

Figure 2.2: Photograph of the study site showing vegetation growth during a) winter 2014 and b) summer 2014/2015.

A small number of vertebrates were present during the trials consisting of grazing animals, such as sheep and goats, and a few bird species, such as a small flock of crowned guineafowl (*Guttera puchernai* Hartlaub), francolins (*Francolinus swainsonii* (Smith)) and greater striped swallows (*Hirundo cucullata* Boddaert). Disturbance by these vertebrate species was limited by placing the above-ground carcasses within wire cages, as well as placing wire cages over the graves.

2.2. Preparation

The seasonal trials were created in order to simulate crime scene cases involving burial during extreme winter and summer conditions. Seven frozen pig carcasses (*Sus scrofa* Linnaeus) were utilised per trial as an internationally accepted substitute for human cadavers (Catts & Goff 1992). The frozen pig carcasses utilised for the winter trial were obtained already euthanised from the Animal Experimentation Unit, located on the

University of the Free State grounds, and thawed before being placed in the field. The pigs utilised for the summer trial were brought in and euthanised by single euthanasia injection ("Euthapent" (Kyron Laboratories (Pty) Ltd) – Pentobarbitone sodium, 1-2ml/kg), and subsequently frozen and thawed before the trial. The Animal Ethics Committee of the University of the Free State had approved this procedure for the summer trial (Appendix A). The winter trial euthanasia had already been approved during a separate study conducted by the Animal Experimentation Unit. Despite concerns by various researchers regarding the freezing, and subsequent thawing of carcasses, and the impact it may have on arthropod activity and decomposition, previous studies by Payne (1965) and Grisbaum *et al*. (1995) found that frozen-thawed carcasses did not have an effect on arthropod succession above-ground. Bugajski *et al*. (2011) also found that frozen-thawed carcasses had no significant impact on the life stages of arthropods or decomposition in burial studies.

Studies by Lane (1975), noted the effects some euthanising agents had on arthropod succession. However, due to the same agent being used in both seasonal trials, any possible effects were rendered irrelevant. The use of this agent allows results from this project to be applicable to human trials as the effects of the chemical is minimal on colonisation and decomposition and the results can be directly applied to real crime scene cases. In accordance with recommendations from Catts & Goff (1992), all carcasses used were within the weight range of 23 kg - 40 kg (Table 2.1). This provided an acceptable weight range that would not affect decomposition and arthropod succession (Hewadikaram & Goff 1991).

Table 2.1: Weight range of the pig carcasses utilised for the two seasonal studies.

Season	Weight range of carcasses
Winter	24 to 37 kg
Summer	23 to 35 kg

Six graves, each 60 cm deep, were dug using an industrial excavator one day before the start of each trial (Figure 2.3). Each grave was 1.6 x 1.2 m in size and the graves were spaced out randomly with at least 20 m between each grave to prevent migration of arthropods. A control carcass was placed above-ground 50 m away from the nearest grave

in a welded wire mesh cage (1.6 x 0.9 x 0.9 m) for both winter (Figure 2.4) and summer (Figure 2.5). To ensure similar results, two different localities within the field, with similar vegetation and ground level, were utilised for the winter and summer trials.

Figure 2.3: Industrial excavator used to dig the graves.

Figure 2.4: Layout of the graves (Red, numbers 1 - 6) and control carcass (Blue, denoted by C) for the 2014 winter trial (Image altered from Google Earth, accessed 09 November 2015).

Figure 2.5: Layout of the graves (Red, numbers 1 - 6) and control carcass (Blue, denoted by C) for the 2014/2015 summer trial (Image altered from Google Earth, accessed 09 November 2015).

All carcasses were wrapped in 25 mm chicken wire (1.0 x 1.5 m) to allow for easy weighing (Figure 2.6). All carcasses were then placed in the graves with their heads facing north, in a north-south orientation, and legs facing west (Figure 2.7). This was to ensure consistency in photoperiod exposure as well as to prevent pseudoreplication during the study. Placement of the carcasses in the field was designated as day 0. Both the control carcass and the graves for each season were fully exposed to sunlight for the entirety of the trial.

Figure 2.6: Euthanised pig wrapped in chicken wire to be placed in a grave.

Figure 2.7: Carcass placed in a grave with head facing north and legs facing west.

During the initial preparation of the graves, 20 soil samples were taken from each grave at random depths and sorted through to determine what arthropod species were present before burial. These specimens were then preserved in 70% ethanol and identified down to species level, when possible, and recorded. Additional soil samples were taken from each grave during winter and summer at 30 cm and 60 cm depths. Soil composition of each grave was then determined from these samples and compared (Table 2.2). Determination of soil type was based upon texture, physical characteristics and water retention.

Table 2.2: Soil composition of graves during winter and summer at 30 cm and 60 cm depths (dominant soil type stated first).

Season	Depth	Grave 1	Grave 2	Grave 3	Grave 4	Grave 5	Grave 6
Winter	30 cm	Sandy soil	Sandy-shale mix	Sandy-clay mix	Sandy-clay mix	Sandy-clay mix	Clay-loam mix
	60 cm	Sandy-shale mix	Clay-shale mix	Shale-sand mix	Clay-shale mix	Shale-sand mix	High clay-loam mix
Summer	30 cm	Clay-sand mix	Clay-loam mix	Clay-loam mix	Clay-sand mix	High clay-loam mix	Clay-loam mix
	60 cm	Clay-loam mix	High clay-loam mix	High clay-loam mix	Clay-loam mix	High clay-loam-shale mix	High clay-loam mix

Placement of carcasses occurred on the solstice days of the two seasons, and these days were designated as day 0. Both trials then began a day after the solstice (day 1) of their respective season (21 June 2014 for winter and 21 December 2014 for summer) to ensure proper seasonal temperatures had been reached before commencement of each trial. Each trial was conducted for a total of 60 days (22 June 2014 till 20 August 2014 for winter, and 22 December 2014 till 19 February 2015 for summer) to allow for comparative analysis between burial and above-ground decomposition and arthropod succession.

2.3. Sampling Methods

2.3.1 *Excavation*

Using the study by Pastula & Merritt (2013) as a guideline, each grave was excavated using conventional tools on predetermined dates for both seasons (Table 2.3). Each carcass was excavated once during the 60 day periods. This provided observations on both short- and long-term effects of burial on decomposition and arthropod succession. After each excavation, the carcasses were reburied at 60 cm in order to dispose of the carcasses and allow decomposition to continue.

After its initial excavation on day 3 of the study, grave one was then excavated every third day over the 60 day period, amounting to a total of 20 excavations for grave one (Table 2.3). This was done to measure the effect of disturbance on decomposition and arthropod succession of buried cadavers, as well as provide adequate insight into temperature fluctuations below-ground during summer and winter. The carcass of grave one was reburied at 60 cm after each excavation.

Table 2.3: Layout of excavation times of graves during the winter and summer trials (Additional grave one excavation times marked in red).

Day of excavation	Grave 1	Grave 2	Grave 3	Grave 4	Grave 5	Grave 6
Day 3	**X**	-	-	-	-	-
Day 6	**X**	-	-	-	-	-
Day 7	-	**X**	-	-	-	-
Day 9	**X**	-	-	-	-	-
Day 12	**X**	-	-	-	-	-
Day 14	-	-	**X**	-	-	-
Day 15	**X**	-	-	-	-	-
Day 18	**X**	-	-	-	-	-
Day 21	**X**	-	-	**X**	-	-
Day 24	**X**	-	-	-	-	-
Day 27	**X**	-	-	-	-	-
Day 30	**X**	-	-	-	**X**	-
Day 33	**X**	-	-	-	-	-
Day 36	**X**	-	-	-	-	-
Day 39	**X**	-	-	-	-	-
Day 42	**X**	-	-	-	-	-
Day 45	**X**	-	-	-	-	-
Day 48	**X**	-	-	-	-	-
Day 51	**X**	-	-	-	-	
Day 54	**X**	-	-	-	-	-
Day 57	**X**	-	-	-	-	-
Day 60	**X**	-	-	-	-	**X**
Key: X : Excavation - : No excavation						

2.3.2 *Temperature and Environmental Factors*

A variety of temperatures were recorded during both seasonal trials. A total of six temperatures were recorded during each excavation, namely:

- Grave surface temperature (taken 2 cm below soil surface)
- Soil temperature above buried carcass
- Soil temperature on dorsal side of buried carcass
- Soil temperature on ventral side of buried carcass
- Body temperature of buried carcass
- Soil temperature beneath buried carcass

Each of the temperatures was recorded at midday using a digital probe thermometer. During the excavations the body temperature of the above-ground control carcass was recorded every third day for comparative analysis. To acquire proper body temperatures, the probe of the thermometer was inserted into the body cavity and, once readings had stabilised, the temperature was taken. Ambient temperature was recorded on a daily basis at midday to obtain maximum temperatures experienced in the field during the trial periods. These readings were then compared to midday ambient temperatures obtained from two weather service stations in the Bloemfontein region to compare differences in ambient temperature experience in the Bloemfontein area. One station was located in the Universitas area near the trial site ($29^0$06'46"S; $26^0$10'14"E, ±1437m above sea level) and the other at the Bram Fischer Airport ($29^0$06'00"S; $26^0$18'00"E, ±1353m above sea level). The weather station within the Universitas area was located in a residential area with various housing and vegetation surrounding it. The airport weather station was located on the airport tarmac within an open field area. Rainfall was measured for the winter and summer trial period on a daily basis using a store bought rain gauge which was mounted in the field.

2.3.3 *Carcass Decomposition and Arthropod Sampling*

Daily observations regarding arthropod presence were made on the control carcass as well as the grave surfaces. Observations pertaining to the stage of decomposition of the control and buried carcasses were described based on observations and characteristics by Payne (1965) and Anderson & Van Laerhoven (1996), in regards to their physical appearance, extent of bloat, amount of decomposition fluids, odour, and visibility of internal organs. Sampling of arthropods from the buried carcasses occurred during excavation days if present. Arthropods were sampled from the above-ground control carcass every three days at midday according to guidelines put forward by Lord & Burger (1983). In order to prevent disturbance to arthropod succession small samples of approximately 50 to 80 specimens were taken from the carcasses when possible. Sampled specimens were identified down to species level when possible, and their presence, abundance and distribution on the carcasses were recorded during the seasonal trials. Fungal species analysis was performed by Dr Marieka Gryzenhout from the University of the Free State. Sampled mite species were identified by Dr Louise

Coetzee from the Natural History Museum, Bloemfontein, and Dr Edward Ueckermann from the Agricultural Research Council, Pretoria. Sampled spider species were identified by Dr Charles Haddad from the University of the Free State.

Dipteran larvae and eggs were sampled from the carcasses, when available, and half of the sampled specimens were reared to adulthood for identification purposes. The remainder were placed in warm water (90^0C) and then preserved in 70% ethanol for analysis. Dipteran immatures and adults were identified using keys provided by Brink (2009 *unpublished*) and Zumpt (1961, 1965a, b, 1972) respectively. Phoridae species were identified by Dr Henry Disney from the University of Cambridge. Forensically important coleopteran species were identified using keys compiled by Almeida & Mise (2009).

2.3.4 *Biomass Loss of Carcass*

Each of the carcasses was weighed before placement in the field for their respective seasonal trial. During the 60 day period each of the buried carcasses was weighed on their excavation day using a hanging scale (Figure 2.8). These weights were recorded to determine biomass loss over increasing periods of minimal disturbance.

Figure 2.8: Hanging scale used to weigh the carcasses to determine biomass loss.

Soil was removed from the buried carcasses before weighing during excavations to ensure adequate results for comparison to the original weight of each carcass before their initial burial. Soil was removed by scraping excess soil from the carcass. After its first excavation, grave one was excavated every third day and during these excavations the carcass was weighed to determine biomass loss during high levels of disturbance. The above-ground control carcass was weighed in conjunction with grave one's excavations.

2.4 Statistical Analysis

Presence and absence data of species accumulated during the two seasonal trials was analysed per season to determine the association between above-ground and below-ground carcasses during the two seasons. All analyses were performed using SAS/STAT (version 13.1; SAS Institute Inc.; Cary, North Carolina).

A simple correspondence analysis was carried out on the contingency tables, formed by the data provided from the carcasses, by species cross classification. This analysis provided ordination of the species present on the carcasses during both seasons and enabled interpretation of taxa clustering. The analysis was carried out using SAS procedure CORRESP (SAS 2013).

Seasonal presence and absence data of the various species on the control carcasses versus the corresponding buried carcasses for each season was cross tabulated in 2x2 tables. Association between the controls and buried carcasses for both seasons was analysed using Fisher's exact test, and the relevant P-value was reported. Furthermore, Pearson's correlation coefficient, for the correlation between the seasonal controls and graves, was reported. This analysis was carried out using SAS procedure FREQ (SAS 2013).

2.5 Parallel Study

This study ran concurrently with another burial study conducted by van der Merwe (2016 *unpublished*), entitled: "The identification of Diptera of the grave and their succession patterns during winter and summer in central South Africa, with reference to forensic applications". Observations and results from this parallel study were used as supporting material and are indicated in the text as such.

CHAPTER 3

Results and Discussion

SECTION 3.1:

A comparison of seasonality on above- and below-ground arthropod succession and decomposition: A winter trial

This section reports on the results obtained during the winter trial. In previous winter studies decomposition and arthropod succession has been found to be reduced in terms of rate and abundance (Kelly 2006 *unpublished*; Gilbert 2014 *unpublished*). Main causative factors for such reductions correlate with weather conditions experienced during winter seasons, along with geographical conditions (Byrd & Castner 2010). Anthropogenic interference becomes a concern during such studies as being able to disrupt arthropod succession and colonisation, giving rise to subjective results, especially during the winter season (De Jong & Hoback 2006). Temperatures recorded during the trial are presented for both above- and below-ground situations and compared. Utilising characteristics and groupings provided by Payne (1965) and Anderson & Van Laerhoven (1996), the various stages of decomposition of the carcasses during the winter trial were recorded. Arthropod succession was categorised according to presence and abundance while aggregation analyses were represented graphically. Statistical analyses are presented in terms of ordination as well as association and correlation between carcasses.

3.1.1 Ambient Temperatures, Grave Temperatures and Rainfall

Temperature data recorded over the winter trial period provided insight into temperature fluctuations above- and below-ground. Midday ambient temperature at the study site was compared to weather data recorded at the Universitas and Bram Fischer Airport weather stations to note the differences in ambient temperature experienced within the Bloemfontein area (Figure 3.1.1). Midday ambient temperatures recorded by the weather stations over the 60 day trial period were usually lower in comparison to the temperatures recorded at the study site. This can be attributed to the weather stations being located in built-up areas, particularly the Universitas area, which provided a buffering effect from environmental factors. The study site, in comparison, was open and exposed to high amounts of solar radiation and wind. The differences recorded illustrate how ambient temperature differs within the Bloemfontein area. This confirms the practice of adjusting temperature accordingly at a crime scene during investigations.

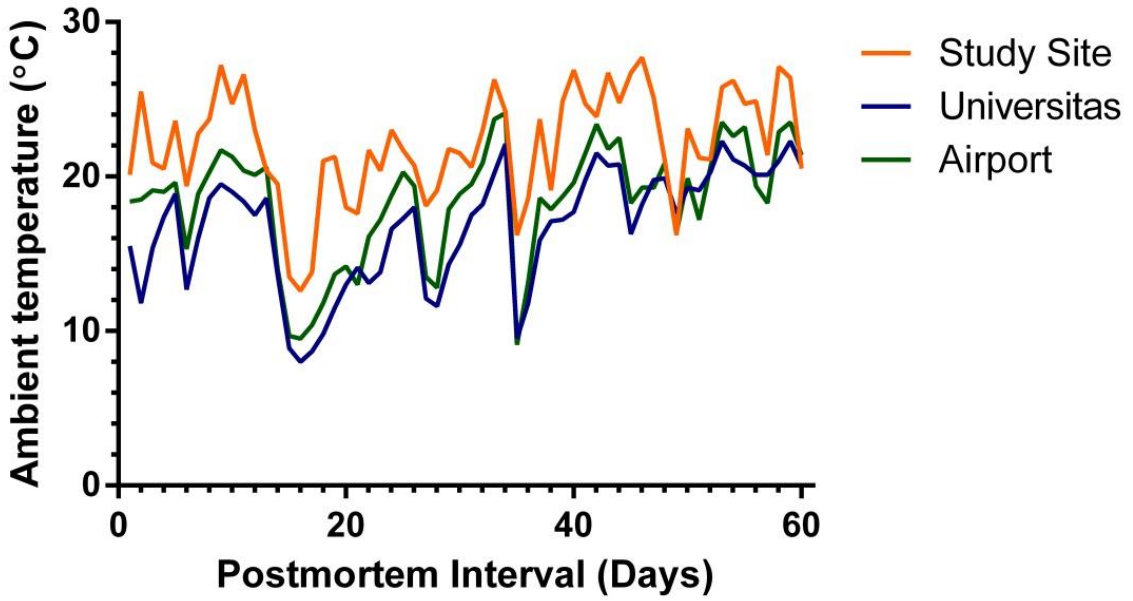

Figure 3.1.1: Midday ambient temperatures recorded at the study site and the Universitas and Bram Fischer Airport weather stations during the winter trial in 2014.

Rainfall throughout the duration of the winter trial was minimal, with a total of 8.5 mm recorded at the study site (Figure 3.1.2). Dry winds and a number of cold fronts experienced during the trial ensured that any rainfall that did occur did not penetrate the soil sufficiently to reach the carcasses in the graves.

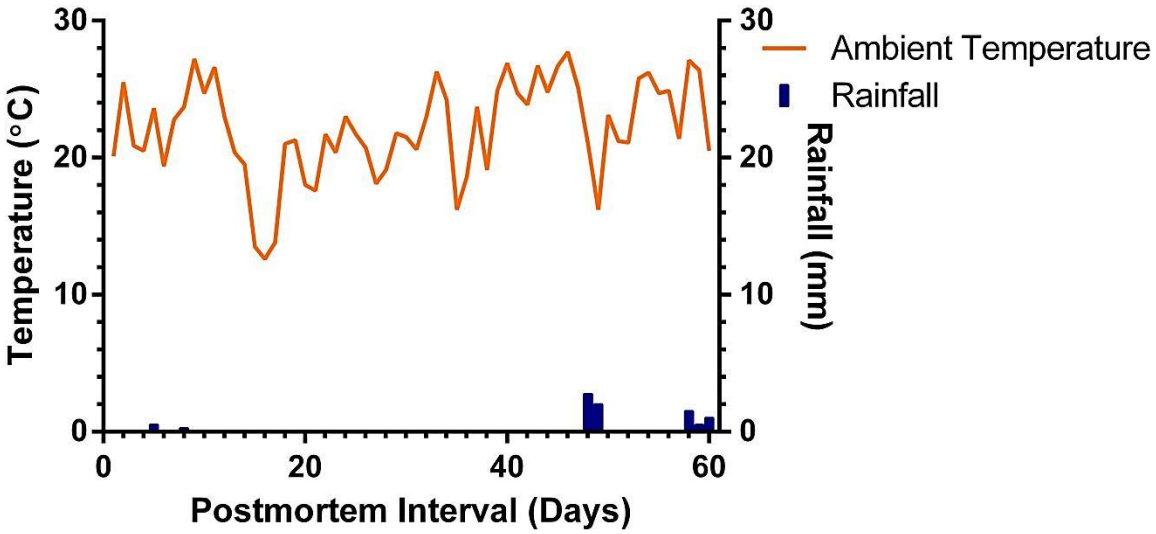

Figure 3.1.2: Rainfall and midday ambient temperatures at the study site during the winter trial in 2014.

Internal grave temperatures recorded from the disturbed grave one were often markedly lower compared to ambient and surface temperatures, with a few exceptions occurring (Figure 3.1.3). Ambient and surface temperatures fluctuated over the 60 day period, while the majority of internal grave temperatures for the disturbed grave one remained within the temperature range of 10°C to 20°C. This data provided insight into the temperature ranges experienced in a grave of 60 cm depth during disturbance.

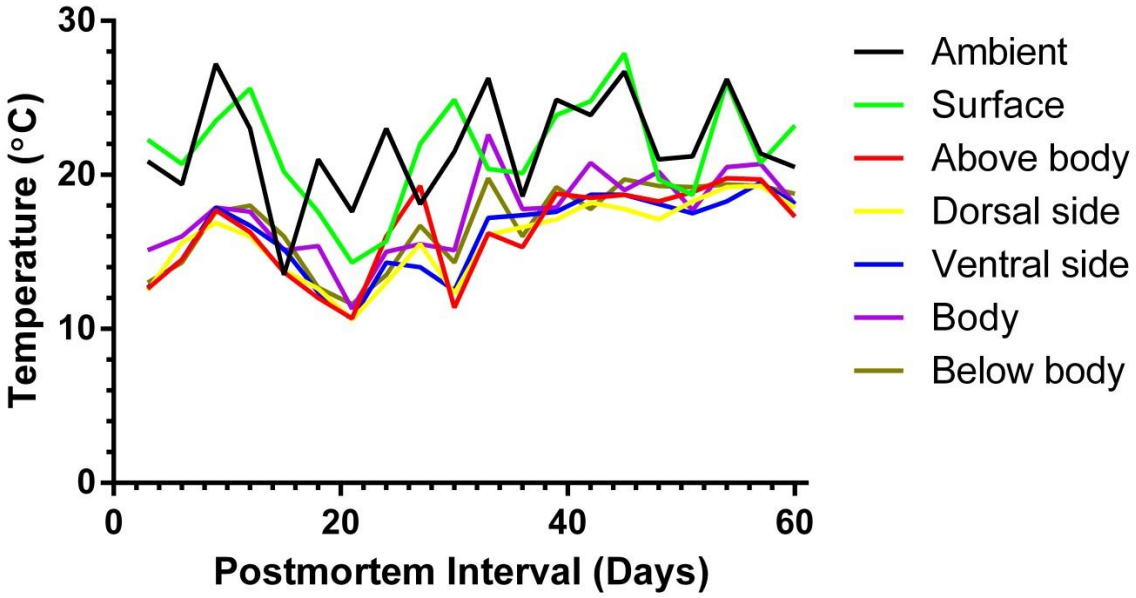

Figure 3.1.3: Grave temperatures of the disturbed grave one in winter (taken every third day), mapped with midday ambient temperatures as recorded during the 2014 winter trial.

Temperature of the control carcass was also taken every third day over the trial period (Figure 3.1.4). Variations in the temperature of the control carcass, compared to the disturbed grave one carcass, show a higher temperature range for the control carcass during most of the trial period. The control carcass was seen to reach temperatures lower than the disturbed grave one carcass on days 15, 18 and 21. On these days low ambient temperatures were recorded and cloud cover was prominent. Fluctuations in the control carcass temperatures mostly corresponded with fluctuations in ambient temperature during the trial period. However, changes in cloud cover during trial days, along with differing wind speeds, must be taken into consideration when determining factors affecting carcass temperatures of the control. In comparison to the disturbed grave one carcass, the control carcass was subjected

to larger temperature fluctuations. This is due to the fact that the control carcass was completely exposed to weather conditions throughout the trial.

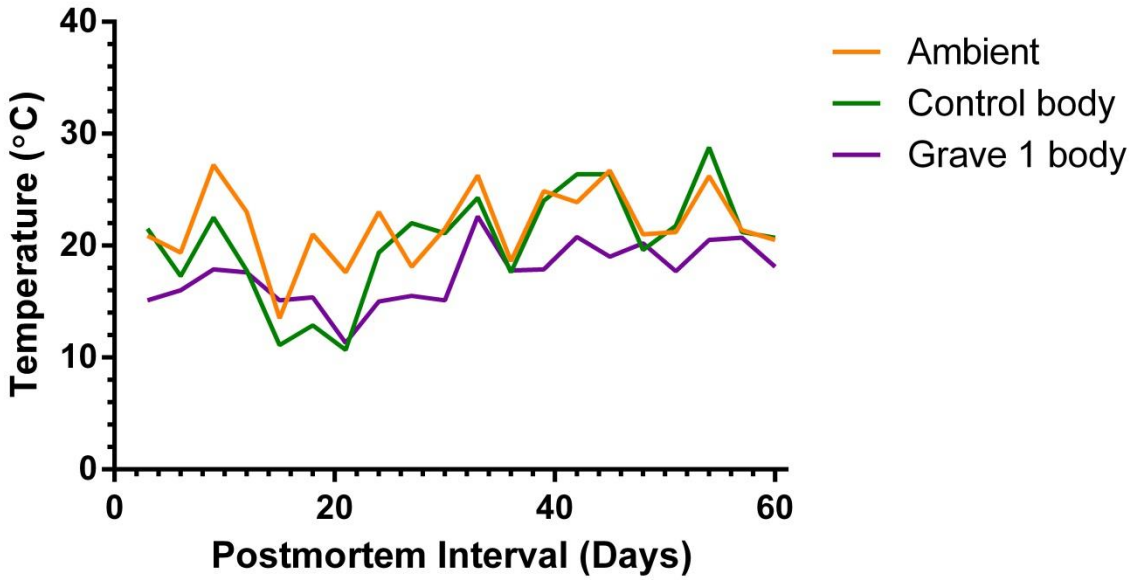

Figure 3.1.4: Temperatures recorded every third day from the control carcass and the disturbed grave one carcass during the 2014 winter trial, mapped with winter ambient temperatures.

Internal grave temperatures taken from the undisturbed graves during excavations showed a decrease in temperature from the surface to the bottom of the grave (Figure 3.1.5). Solar radiation became the main causative factor for surface temperature readings on excavation days, due to each grave being fully exposed to sunlight. Solar radiation penetrates only a small layer of soil and in turn transfers heat within the first 5 cm layer (Lal 2006). Due to initial digging of the graves a loss in vegetation occurred, which caused any grass species present to be removed and the surface of the graves to become exposed. A loss of vegetative cover causes an increase in surface temperature due to vegetation no longer being able to intercept significant amounts of solar radiation (Lal & Shukla 2004). As depth increased marked decreases in temperature were seen for each grave (Figure 3.1.5). Body temperatures of the carcasses in the graves, however, were generally higher than the surrounding soil (Figure 3.1.5) due to anaerobic bacterial action and heat conservation caused by soil compaction. Internal grave temperatures (Figure 3.1.5) remained within a similar temperature range as the disturbed grave one (Figure 3.1.3), with the majority of internal temperatures, from all undisturbed graves, recorded within the range of 12°C to 18°C. Some graves

contained clay soils which are able to become easily compacted when moisture is present. Leakage of decomposition fluids from the carcasses may have led to compaction of soil around them, effectively sealing them and contributing to heat conservation.

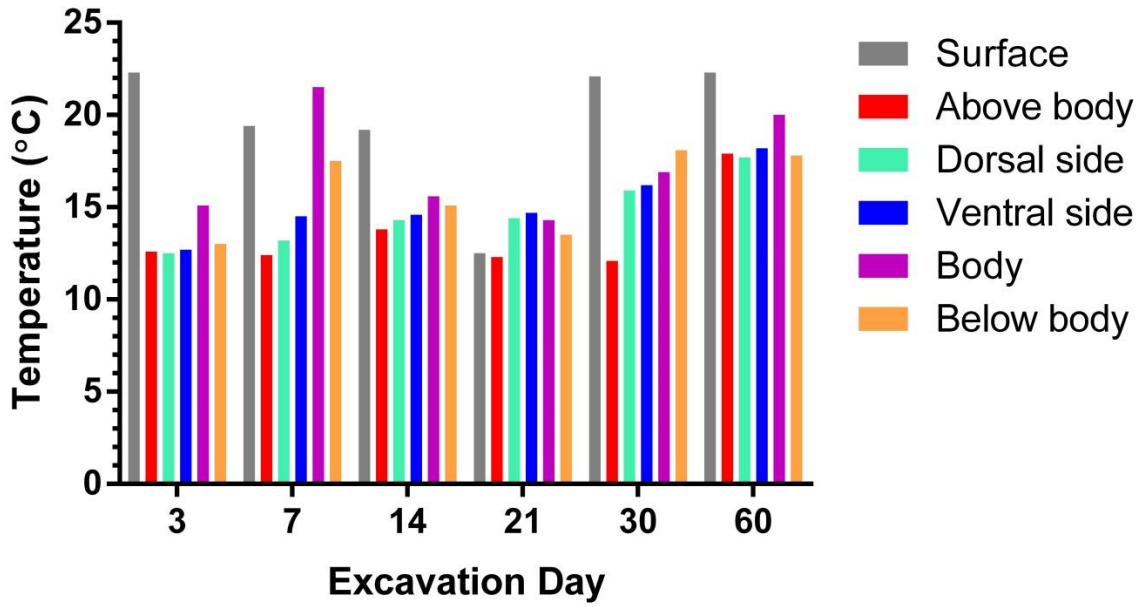

Figure 3.1.5: Grave temperatures of undisturbed graves, recorded during excavations, during the 2014 winter trial.

3.1.2 Carcass Decomposition

The rate of decomposition of the carcasses was slow due to low ambient temperatures and internal grave temperatures. Only three stages of decomposition were noted for the 60 day trial period for both above- and below-ground (Table 3.1.1).

1. *Fresh Stage*

   After placement in the field, the above-ground control carcass remained in the fresh stage until day 11 of the trial while the below-ground fresh stage lasted until only day 8 in grave one and two (Figure 3.1.8). A few adult dipteran species were recorded on the control carcass and no immatures were seen during the fresh stage both above- and below-ground. The carcasses then slowly transitioned into bloat.

2. *Bloated Stage*

   During the bloated stage of decomposition the control carcass inflated partially, but not significantly enough to be characterised as “balloon-shaped”. Below-ground, however,

buried carcasses did not experience a form of inflation that would make characterisation of the bloat significantly noticeable. Therefore, other characteristics, such as presence of decomposition fluids and absence of putrefaction, were used in order to identify this stage. This non-occurrence of inflation deviates from characteristics described by Payne (1965) and Anderson & Van Laerhoven (1996), bringing into question the usage of certain characteristics in below-ground decomposition. Three separate periods of bloat were noted during the trial. The disturbed grave one carcass experiencing bloat from day 12 until day 27, the control carcass was in bloat from day 14 until day 20, and the undisturbed buried carcass of grave three (excavated on day 14) underwent bloat possibly from the same day as the disturbed grave one carcass until day 20 (Figure 3.1.8).

Various adult arthropods were seen on the control carcass during the bloated stage but no larvae were noted either above- or below-ground. Egg clusters were seen beneath the arms of the control carcass and in the oral cavity, indicating oviposition by dipteran species, but no hatching had yet occurred due to the cold weather conditions.

Discolouration of the carcasses was seen during the various bloated stage periods, with more extensive discolouration appearing on the control carcass. Buried carcasses, while not undergoing as extensive discolouration, experienced increased fungal growth as the bloated stage progressed to active decay (Figure 3.1.6).

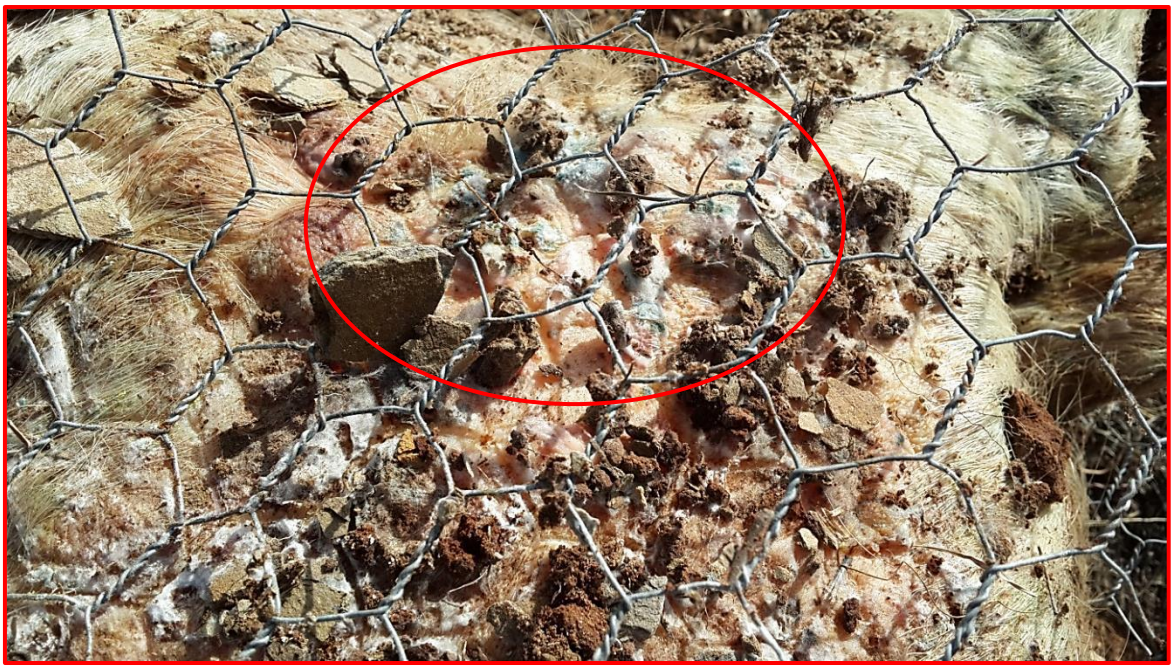

Figure 3.1.6: Fungal growth occurring on the carcass of grave three (day 14, 05/07/2014) during the winter trial.

Table 3.1.1: Summarisation of decomposition stages experienced during the 2014 winter trial for both the above- and below-ground carcasses, as well as most dominant arthropods present during these stages.

Stage of decomposition	Arthropods present
FRESH • Above-ground: Day 0 to 11 • Below-ground: Day 0 to 8 • No odour present • Slight discolouration on neck and belly of some carcass	**Diptera** Calliphoridae: *Lucilia* spp. (adults) (control only) *Chrysomya albiceps* (adults) (control only) *Chrysomya chloropyga* (adults) (control only)
BLOAT • Above-ground: Day 14 to 20 (Transition, day 12 to 13) • Below-ground: Day 12 to 20 (Transition, day 9 to 11) • Beginning of stage characterised by inflation of body due to build-up of gases within carcass • Discolouration of body on neck, belly and hindquarters • Bodily fluids seen to leak out of body through major orifices such as mouth, anus and ears on control carcass • Audible release of gas heard during later days of stage on control carcass • Oviposition by one dipteran species noted during this stage on control carcass • Extent of inflation not as prominent below-ground	**Diptera** Calliphoridae: *Lucilia* spp. (adults & eggs) (control only) *Chrysomya albiceps* (adults) (control only) *Chrysomya chloropyga* (adults) (control only) Muscidae: *Muscina stabulans* (adults) (control only) *Musca domestica* (adults) (control only) Sarcophagidae: *Sarcophaga* sp. (adults) (control only) Phoridae: *Megaselia scalaris* (adults) (control only) **Coleoptera** Dermestidae: *Dermestes maculatus* (adults) (control only) Histeridae: *Macrolister* sp. (adults) (control only)
ACTIVE DECAY • Above-ground: Day 26 to 60 (Transition, day 21 to 25) • Below-ground: Day 21 to 60 • Strong odour of decay present • Skin of control carcass took on a leathery appearance and texture • Large amount of decomposition liquids present beneath both buried and non-buried carcasses • Adipocere formation noted beneath buried bodies • Slight deflation of control carcass towards day 60 • Most dominant arthropods on control carcass noted to be coleopteran species with *Dermestes maculatus* larvae being highly prominent • Skin slippage seen on buried carcasses • Large amounts of fungal growth noted on buried carcasses • Most dominant arthropods below-ground noted to be dipteran species, particularly Phoridae species	**Diptera** Calliphoridae: *Lucilia* spp. (adults) (control only) *Chrysomya albiceps* (adults) (control only) *Chrysomya chloropyga* (adults) (control only) Muscidae: *Hydrotaea* sp. (adults) (control only) *Muscina stabulans* (adults and maggots) (control & graves) *Musca domestica* (adults) (control only) Sarcophagidae: *Sarcophaga* sp. (adults and maggots) (control only) Phoridae: *Megaselia scalaris* (adults and maggots) (graves only) *Conicera tibialis* (adults and maggots) (graves only) Sphaeroceridae: *Leptocera* sp. (adults) (graves only) **Coleoptera** Dermestidae: *Dermestes maculatus* (adults and larvae) (control only) Cleridae: *Necrobia rufipes* (adults) (control only) Histeridae: *Macrolister* sp. (adults) (control only) *Euspilotus* sp. (adults) (control only) **Scolopendromorpha** Scolopendridae: *Cormocephalus* sp. (graves only)

3. *Active Decay*

Active decay dominated the decomposition process time frame. The first carcass recorded as being in active decay was from grave four on day 21 of the trial (Figure 3.1.8), with the control carcass entering active decay on day 26. The disturbed grave one carcass only reached active decay on day 42, showing the effect of disturbance on below-ground decomposition. The presence of dipteran larvae on carcasses during this stage indicated a noticeable delay in colonisation by Diptera. However, the presence and activity by other forensically important arthropods was prominent due to their abundance, particularly the occurrence of the coleopteran species *Dermestes maculatus* De Geer on the control carcass.

Low ambient temperatures and dry winds caused the control carcass to take on a leathery appearance and texture during active decay, becoming partly mummified. The carcasses of grave four (day 21) to six (day 60), on the other hand, remained moist despite a lack of soil moisture, and during later excavations skin slippage and adipocere formation occurred. Such characteristics are common with burial cases (Fiedler & Graw 2003; Forbes *et al*. 2005a). Fungal growth on grave carcasses four (day 21) to six (day 60) showed increased growth with large numbers of *Penicillium* Link species being present (Figure 3.1.7) as identified by Dr Marieka Gryzenhout. Such fungal growth has been recorded on buried carcasses during other similar studies (Suzuki 2001; Carter *et al*. 2007).

Dipteran larvae on the control carcass were not seen to migrate away from the carcass due to high competition and predation by *D. maculatus* and other predatory arthropods. As time progressed carcasses began to collapse as continual feeding by arthropod species occurred, albeit slowly. The disturbed grave one carcass, however, decomposed slower, compared to the undisturbed grave carcasses, due to the high level of disturbance caused by the excavations. The absence of arthropods in this grave also contributed to the slower rate of decomposition as no feeding on the carcass occurred during the trial.

Figure 3.1.7: Increased fungal growth on the winter grave four carcass (day 21, 12/07/2014) during decomposition.

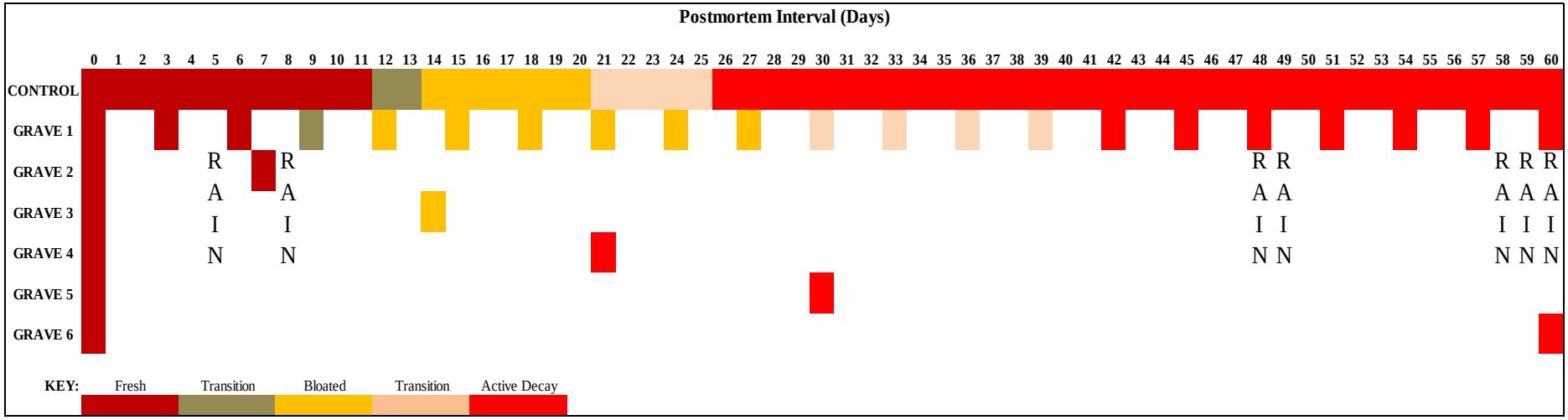

Figure 3.1.8: Stages of decomposition experienced by the carcasses during the 2014 winter trial.

3.1.3 Biomass Loss

Biomass was lost from the control carcass and the disturbed grave one carcass at a steady pace over the winter period (Figure 3.1.9). The control carcass and disturbed grave one carcass lost less than 30% of their biomass at the end of 60 days (Figure 3.1.9). Generally, undisturbed grave carcasses lost biomass at a faster rate than the control and disturbed grave one carcass despite being within an isolated, undisturbed environment. Of interesting note is the fact that the carcass of grave six (day 60) retained more than 95% of its original biomass at the end of the 60 day period, despite being undisturbed for the longest period of time (Figure 3.1.9). Soil composition of grave six was noted to be a mixture of clay and loam which caused the carcass to retain fluids and thus prevented substantial biomass loss. Despite being recorded as being in active decay, the carcass of grave six was only in early active decay. This stage was described by deflation of the body not yet occurring and large amounts of putrefaction still present. This corresponds with less biomass having been lost by the grave six carcass at the end of the trial period. Other graves had a soil composition of mainly shale and sand, allowing leakage of body fluids and, therefore, a higher biomass loss during decomposition. The shale and sand composition of these graves also provided access to specific arthropods at an early stage due to air pockets and gaps present in the soil.

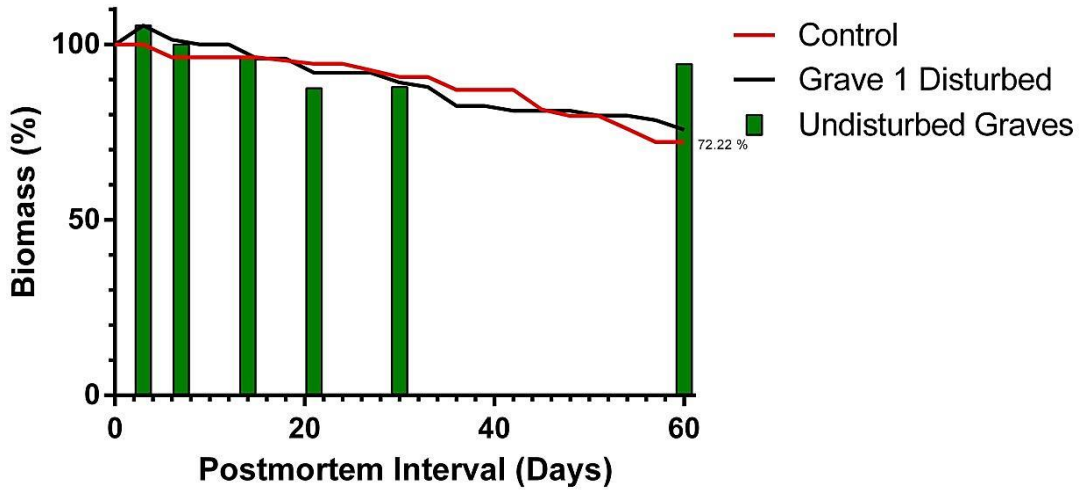

Figure 3.1.9: Rate of biomass loss from carcasses during the 2014 winter trial.

The disturbed grave one carcass showed an interesting occurrence during the first week of decomposition. The biomass of the carcass was seen to increase by day 3, gaining 5% in body

mass, before entering a phase of continual decrease for the remainder of the trial. This initial increase may have been caused by fluid absorption and retention by the grave carcass from the surrounding soil, effectively causing the weight of the carcass to increase. Occurrence of soil on the body could not have caused such an increase as any soil that was present on the carcass was removed before weighing. The rate of biomass loss from the disturbed carcass of grave one was the slowest overall, despite it following a similar pattern of decline to the undisturbed grave carcasses. Due to the high level of disturbance caused by constant excavation, and a lack of feeding by arthropod species, the carcass of grave one contained slightly more biomass than the control carcass at the end of the trial period.

3.1.4 Arthropod Succession

Before burial of the carcasses, soil samples of each grave contained a number of species already present below ground (Table 3.1.2). The most abundant species belonged to the family Formicidae, being present in five out of the six graves. A single Scarabaeidae larva was sampled from grave six.

Table 3.1.2: Arthropod species present in the 2014 winter graves before burial of the carcasses, sampled on 20/06/2014.

Arthropods present	Grave 1	Grave 2	Grave 3	Grave 4	Grave 5	Grave 6
Coleoptera						
Scrabaeidae:						
Scarabaeidae sp. larva	-	-	-	-	-	**X**
Hymenoptera						
Formicidae:						
Camponotus sp.	**X**	**X**	-	**X**	-	-
Cardiocondyla shuckardi	-	**X**	-	-	**X**	-
Messor capensis	-	**X**	-	-	-	-
Solenopsis globularia	-	-	**X**	-	-	-
Solenopsis punctaticeps	-	-	**X**	-	-	-

During the trial, distinct differences in successional rates and species diversity were noted between above- and below-ground carcasses over the winter period.

1. *Above-ground*

Unlike results obtained from previous studies around the world (Campobasso *et al*. 2001; Wolff *et al*. 2001; Kelly 2006 *unpublished*; Kolver 2009 *unpublished*), dipteran species were not the most dominant on the control carcass during winter. The Calliphoridae species *Chrysomya albiceps* (Wiedemann), *Chrysomya chloropyga* (Wiedemann) and *Lucilia* Robineau-Desvoidy (consisting of *Lucilia sericata* (Meigen) and *Lucilia cuprina* (Wiedemann)) adults were seen around the control carcass during the first week of the trial, but very few attempts at oviposition by these species was recorded. Presence of dipteran species became scattered during the trial period above-ground while abundance remained relatively low (Figure 3.1.10). Despite the presence of several adult dipterans, only individuals from the family Sarcophagidae were noted to successfully colonise the control carcass on day 30 (Figure 3.1.11). Due to their ability to larviposit, the larvae of the sarcophagid species were able to immediately find shelter on the control carcass (Coupland & Baker 1994; Singh & Bharti 2008). This method of colonisation prevents predation of eggs and results in a higher survival rate of immature species individuals.

Coleopteran species dominated the control carcass from day 14 onwards and grew in abundance as time progressed with the Dermestidae species *D. maculatus* (Hide beetles), the Histeridae genera *Macrolister* Lewis and *Euspilotus* Lewis, and the species *Necrobia rufipes* (Fabricius) being the most abundant. Daily records showed changing behavioural patterns and abundance of *D. maculatus* on the control carcass as the trial progressed (Appendix B). Predation by coleopteran species on dipteran immatures was seen during the active decay stage of decomposition, particularly on unhatched egg clusters. Immatures of *D. maculatus* occurred on the carcass from day 37 onwards with different development stages present, signifying that these immatures were feeding on the carcass (Figure 3.1.12). Drying out of the carcass by dry winds and cold temperatures, along with the presence of dipteran immatures, may have attracted coleopteran species to the carcass allowing them to dominate and reproduce in turn. The extent to which coleopteran species, particularly *D. maculatus*, colonised the control carcass highlights the possible role this family can play in determining PMI when Diptera fail to colonise.

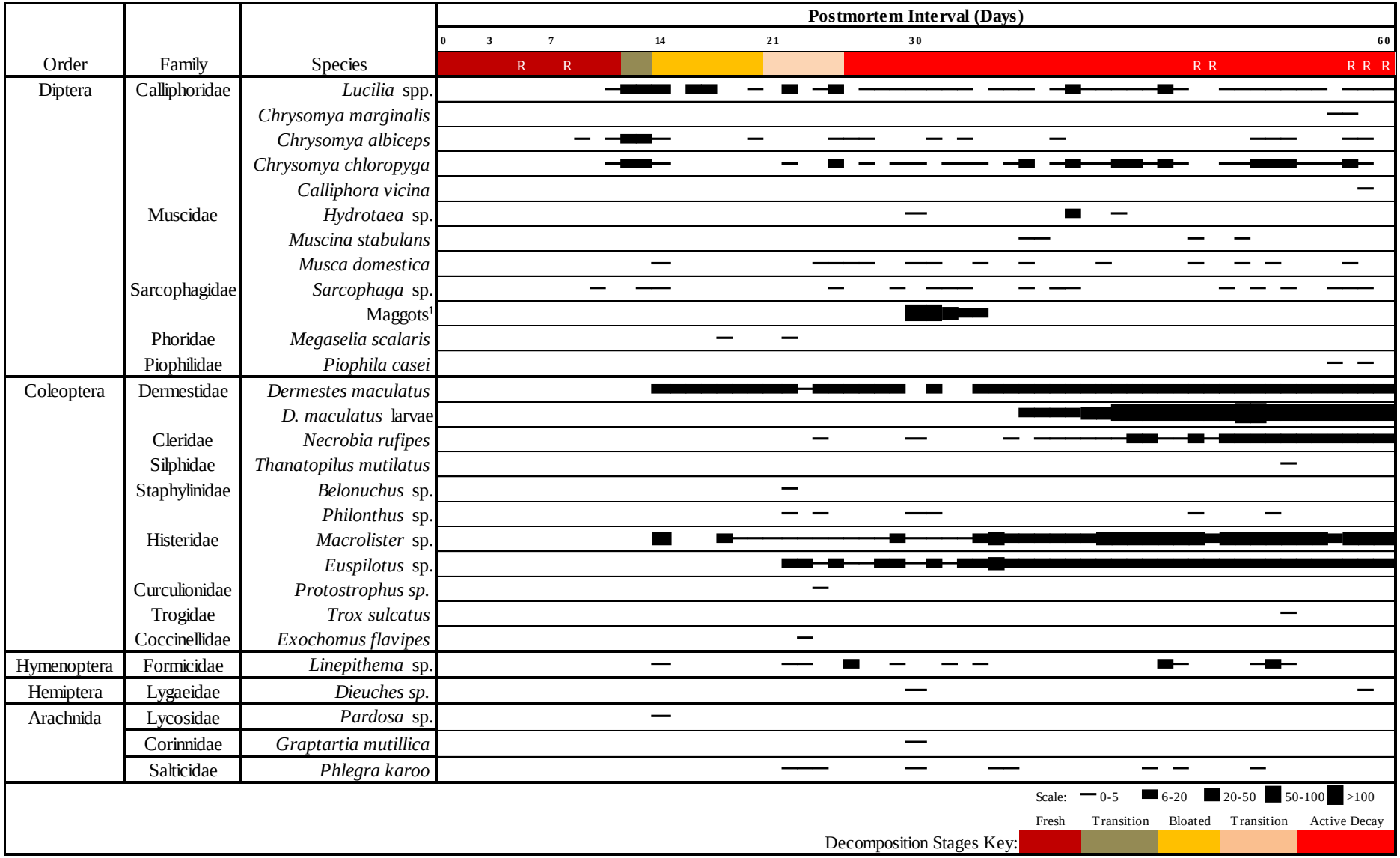

Figure 3.1.10: Arthropod succession on the control carcass during the 2014 winter trial. Maggots[1] - only *Sarcophaga* species. (R - Rainfall).

Figure 3.1.11: Sarcophagidae species larvae present on the 2014 winter control carcass (day 30, 21/07/2014), beneath the arm.

Figure 3.1.12: Larvae and adults of *Dermestes maculatus* feeding on the 2014 winter control carcass (day 39, 30/07/2014).

A small number of predatory arthropod species, specifically Arachnida, appeared on and around the control carcass at random intervals. Predation by these species was seen to occur on dipteran adults, particularly by the arachnid species *Phlegra karoo* Wesolowska (Family: Salticidae) (Figure 3.1.13).

Figure 3.1.13: Arachnid species *Phlegra karoo* preying on a *Chrysomya albiceps* adult at the 2014 winter control carcass (day 30, 21/07/2014).

2. *Below-ground*

Below-ground arthropod succession occurred at a much slower rate compared to above-ground, with fewer species occurring. Various species were found to colonise buried carcasses with dipteran species being the most prominent (Figure 3.1.14). Dipteran specimens were first sampled in grave four on day 21 of the trial, indicating that colonisation possibly occurred between day 14 and 21 as no colonisation was seen in grave three (day 14). The two Phoridae species *Megaselia scalaris* (Loew) and *Conicera tibialis* were recorded as being the most prominent species on buried carcasses during winter (Figure 3.1.14). These two species are often found on buried carrion (Disney 1994; Campobasso *et al*. 2004; Bourel *et al*. 2004). Large numbers of adults and maggots of Phoridae were seen on the buried carcasses (Figure 3.1.15). Despite their ability to easily enter and leave a grave, adult phorids remained on the buried carcasses after oviposition during the trial. This was indicated by the lack of observations of adults leaving the closed

graves and the presence of empty pupal cases, together with an influx of newly emerged adults, discovered upon excavation. This, however, does not rule out the possibility of new individuals colonising the carcass from outside the grave. Persistence of existing phorid individuals was due to their small size and morphological adaptations which allows them to survive below-ground for extended periods of time (Martín-Vega *et al*. 2011). It is possible that the main factor causing the adults to remain on the carcasses is the availability of an adequate food source to support several generations existing on a single carcass.

A species from the genus *Leptocera* Olivier (Family: Sphaeroceridae) was recorded in large numbers on day 60, but no immatures of this species were found. Some species of this genus are known to be attracted by fungi and actively feed on fungal growth (Buck 1997). With fungal growth prominent on the undisturbed grave six carcass it stands to reason that attraction of this *Leptocera* species was most likely due to the occurrence of fungi on the buried carcass. It has not yet been clarified if this species plays a role in succession (and subsequent PBI estimations) and decomposition of carcasses during the later stages of decomposition, or if it is simply an indicator species.

Of interesting note was the occurrence of larvae of the muscid species *Muscina stabulans* (Fallén) in grave six (day 60). This species has been found to occur on decomposing carrion, both buried and non-buried, and has mainly been found during the later stages of decomposition and on shallow buried carrion at 30 cm (Szpila *et al*. 2010; Mariani *et al*. 2014) and 40 cm (Gunn & Bird 2011). The fact that larvae were found at a depth of 60 cm during the present study indicates the ability of this species to reach a deeply buried carcass and colonise it. It is possible that the adults themselves were unable to reach the buried carcass, ovipositing instead in the top soil of the grave and the hatched larvae were able to burrow down to the carcass. Previous studies have found the larvae of *M. stabulans* capable of burrowing to certain depths (Gomes *et al*. 2006), and this may explain why only larvae, and not adults, of this species were present on the grave six carcass (day 60).

A single predatory arthropod specimen was sampled on day 30 of the trial belonging to the centipede family Scolopendridae. The *Cormocephalus* (Newport) sp. individual, belonging to a well-documented predacious centipede genus (Lawrence 1983), was seen to feed upon phorid larvae on the buried carcass of grave five (day 30). This is indicative of the interactions occurring below-ground during arthropod succession. It thus falls to reason

that predator-prey interactions may influence succession patterns and affect PBI estimation, but to what extent is still unknown.

During the entire winter trial no species were sampled from the disturbed grave one carcass. This indicates the impact of disturbance by burial on arthropod succession. Furthermore, none of the species sampled from before burial of the carcasses were present in the undisturbed graves at their time of excavation, indicating to investigators that they can rely upon arriving arthropods for PBI estimation and can ignore the possibility that those arthropods were already present below-ground at the time of burial.

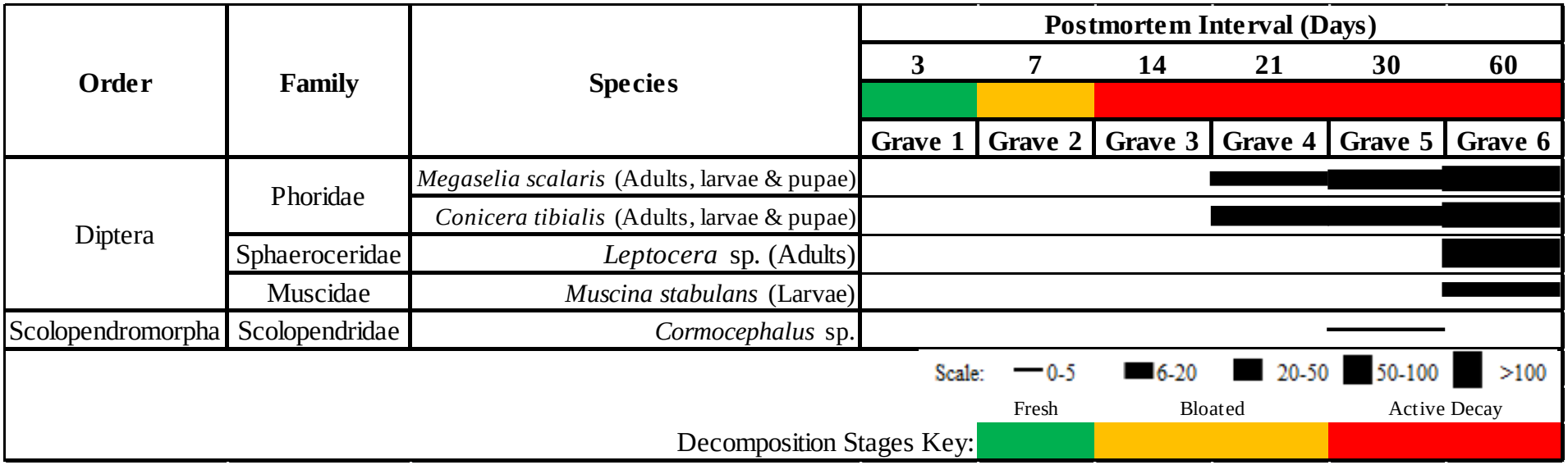

Figure 3.1.14: Arthropod succession on the undisturbed buried carcasses during the 2014 winter trial.

Figure 3.1.15: Phoridae larvae present on the grave five carcass during the 2014 winter trial (day 30, 21/07/2014).

3.1.5 Arthropod Aggregation Sites

Distributions of arthropod masses were recorded daily on the control carcass (Figure 3.1.16) and during each excavation (Figure 3.1.17). Arthropod masses differed in terms of distribution between the control and colonised grave carcasses.

While a number of sites were utilised by dipteran species for oviposition on the control carcass, larvae were noted at only a single site (Figure 3.1.16). The distribution of adult coleopteran species on the control carcass was wide and varied with overlaps occurring, particularly with dipteran oviposition sites. While no primary oviposition sites of *D. maculatus* were noted, the first occurrence of larvae was observed on the stomach of the carcass. During the last days of the trial the distribution of these larvae had spread over the entirety of the control carcass. The remaining coleopteran species, mainly *Macrolister* and *Euspilotus* species and *N. rufipes*, remained mainly beneath the trunk of the body with some individuals migrating onto the top of the head.

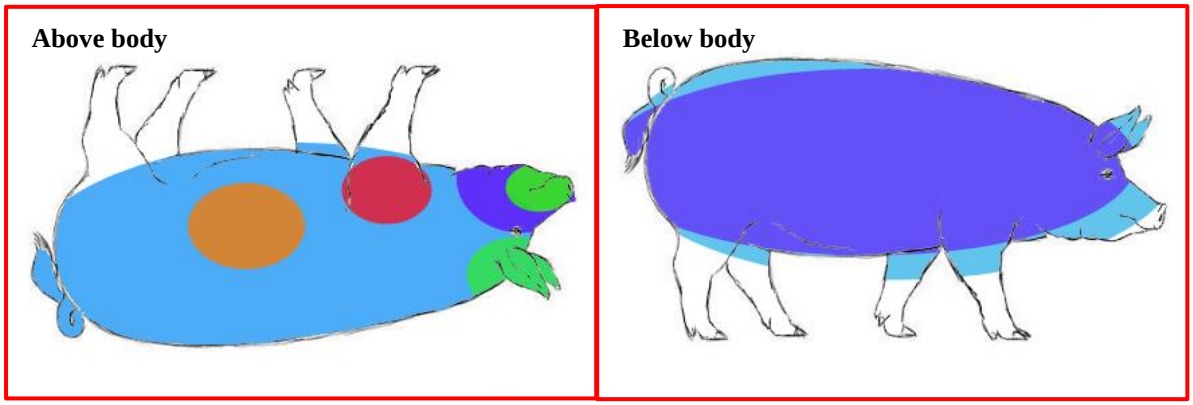

Figure 3.1.16: Arthropod distribution on the 2014 winter control carcass. Green: Non-emerged dipteran oviposition sites; Red: Emerged dipteran oviposition sites; Blue: *Dermestes maculatus* distribution; Orange: First occurrence of *Dermestes maculatus* immatures; Purple: Distribution of remaining coleopteran species.

Distribution on the colonised buried carcasses (Figure 3.1.17) showed only a few specific regions being colonised compared to the wider distribution on the control carcass (Figure 3.1.16). Mainly dipteran species were present on the buried carcasses, apart from an additional single centipede individual occurring in grave five (day 30).

The first immatures present during the trial were from the Phoridae species *M. scalaris* and *C. tibialis* on day 21, with muscid *M. stabulans* larvae occurring later on day 60. These immatures were observed to occur mainly beneath the body around the legs, hooves and stomach of the carcasses (Figure 3.1.17). These areas experienced large amounts of adipocere formation and putrefaction due to high levels of moisture being retained beneath the body (Figure 3.1.18). Adult phorids were recorded in scattered locations on the colonised carcasses but the majority of individuals remained underneath the carcasses. Phoridae pupae were also sampled from beneath the body, around the legs, stomach and hooves, at the sites where larvae were usually seen. Adult *Leptocera* sp. individuals remained beneath the body where moisture levels were high. According to Hofkin (2010), these moisture levels provide a beneficial environment for fungal growth. No immatures of the *Leptocera* sp. were found. *Muscina stabulans* was able to colonise the grave six carcass (day 60), and larvae were found beneath the body around the back legs (Figure 3.1.17). A single centipede individual was sampled on the grave five (day 30) carcass from beneath the body, and was seen to prey on dipteran adults and larvae.

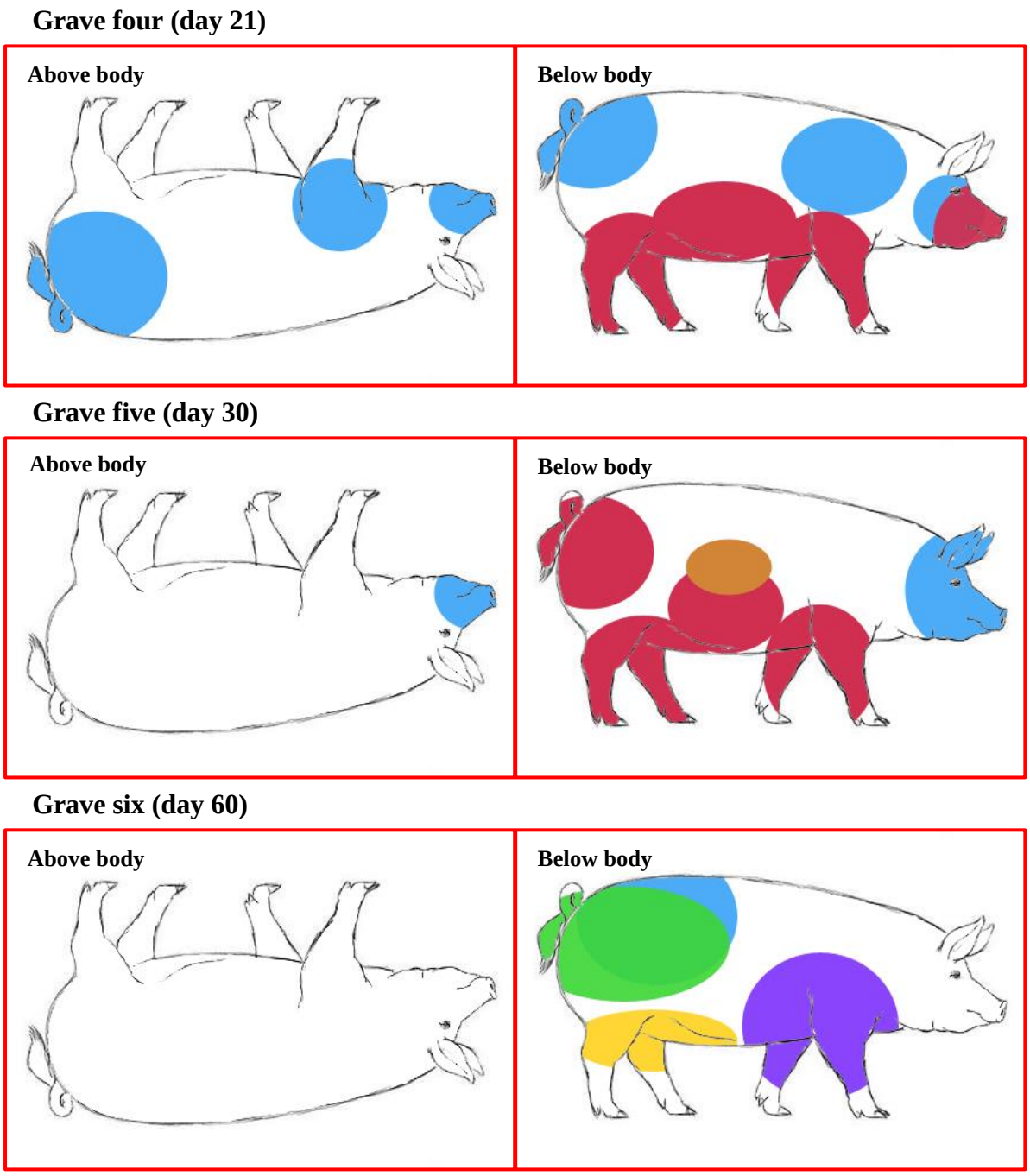

Figure 3.1.17: Arthropod distribution on the colonised winter grave carcasses during 2014**.** Red: Distribution of Phoridae immatures; Blue: Distribution of Phoridae adults; Purple: Distribution of Phoridae pupae; Green: Distribution of Sphaeroceridae adults; Yellow: Distribution of *Muscina stabulans* immatures; Orange: Occurrence of single *Cormocephalus* sp. individual.

Figure 3.1.18: Phoridae larvae present around the legs of the grave six carcass during the 2014 winter trial (day 60, 20/08/2014). Note the large amount of adipocere formation and putrefaction.

3.1.6 Statistical Analysis

Correspondence analysis ordination showed typical clustering of the majority of sampled species around the control carcass from a total of 28 species sampled during the trial (Figure 3.1.19). Due to major clustering of taxa around the control carcass, obscuring species names, a separate list of these species is contained in Table 3.1.3. While graves four (day 21), five (day 30) and six (day 60) showed less species diversity and abundance, a clear separation of species involved in burial cases can be seen, particularly in regards to the two Phoridae species *M. scalaris* and *C. tibialis*. Overlaps of species between the graves and control carcass did occur, with only two species, namely *M. scalaris* and *M. stabulans*, occurring on both above- and below-ground carcasses as indicated by their position in the ordination. It should be noted that only adults of these species were present above-ground compared to both adults and/or larvae being present below-ground.

Results of Fisher's exact test for association between the graves and the control carcass differed, with a P-value of 0.2063 for grave four (day 21), a P-value of 0.0232 for grave five (day 30) and a P-value of 0.0452 for grave six (day 60). This indicates a significant association ($P<0.05$) between the control carcass and graves five and six due to overlaps in

species presence, in regards to *M. scalaris* and *M. stabulans*. A non-significant association (P>0.05) was seen between the control carcass and grave four, with only adults of *M. scalaris* occurring above ground. This can be attributed to fewer species and a lower abundance compared to graves five and six. The association of the remainder of the graves with the control carcass was zero since no species were sampled from graves one, two and three.

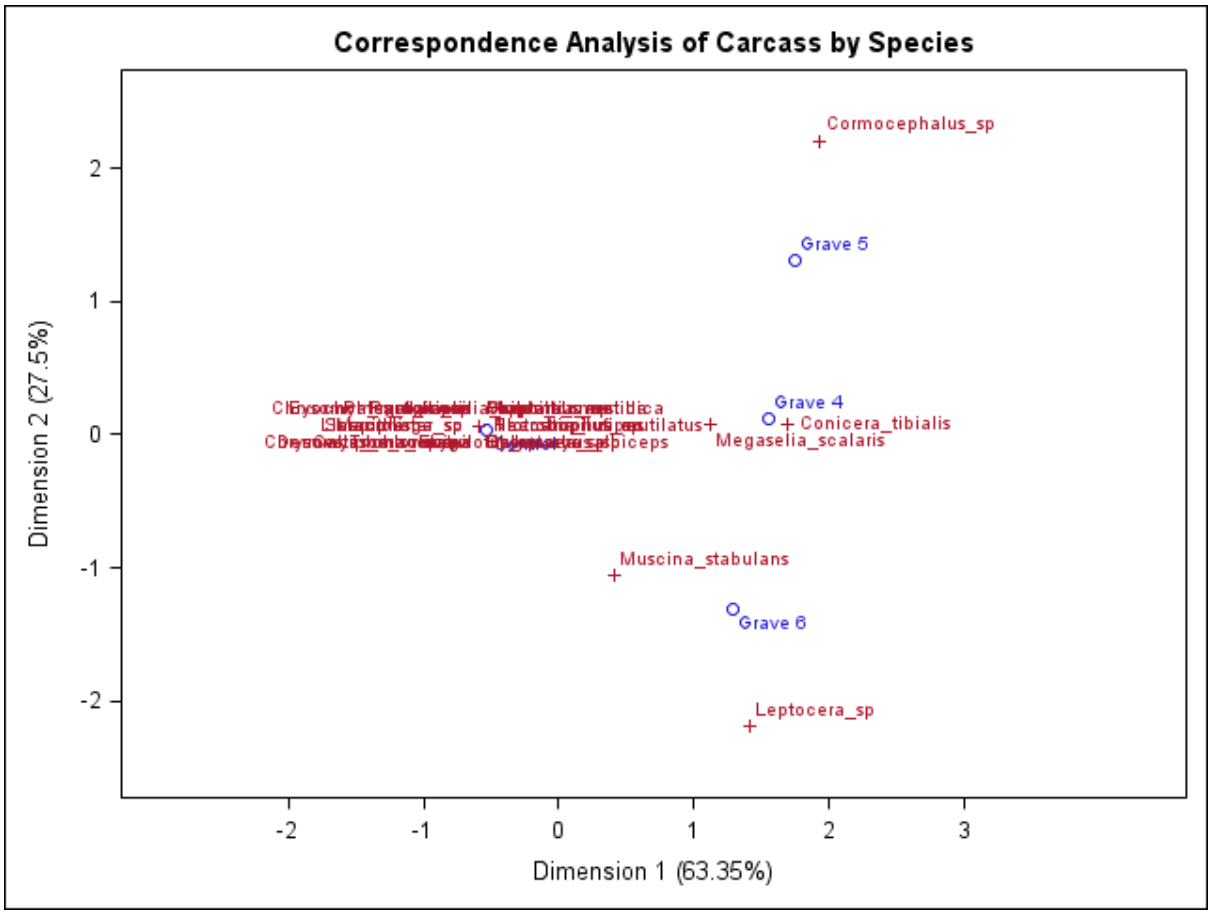

Figure 3.1.19: Correspondence analysis ordination showing clustering of species at the carcasses during the 2014 winter trial.

Pearson's correlation coefficient measures the strength of association between the various graves and the control carcass, with regard to the presence/absence of species. All correlation coefficients were negative, being -0.3523 for grave four, -0.6267 for grave five and -0.5185 for grave six. Once again since no species were sampled on the remainder of the graves the correlation of these graves with the control carcass could not be determined. The correlations between the control carcass and the graves being negative (when they could be calculated) suggests that a species was more likely to be present on a grave carcass if it was absent on the control carcass.

Table 3.1.3: List of species clustered around the 2014 winter control carcass on correspondence analysis ordination.

Order	Family	Species
Diptera	Calliphoridae	*Lucilia* spp.
		Chrysomya marginalis
		Chrysomya albiceps
		Chrysomya chloropyga
		Calliphora vicina
	Muscidae	*Hydrotaea* sp.
		Musca domestica
	Sarcophagidae	*Sarcophaga* sp.
	Piophilidae	*Piophila casei*
Coleoptera	Dermestidae	*Dermestes maculatus*
	Cleridae	*Necrobia rufipes*
	Silphidae	*Thanatopilus mutilatus*
	Staphylinidae	*Belonuchus* sp.
		Philonthus sp.
	Histeridae	*Macrolister* sp.
		Euspilotus sp.
	Curculionidae	*Protostrophus* sp.
	Trogidae	*Trox sulcatus*
	Coccinellidae	*Exochomus flavipes*
Hymenoptera	Formicidae	*Linepithema* sp.
Arachnida	Lycosidae	*Pardosa* sp.
	Corinnidae	*Graptartia mutillica*
	Salticidae	*Phlegra karoo*

3.1.7 General Overview

The rate of decomposition during the winter trial was seen to differ between above- and below-ground carcasses (Figure 3.1.8). The control carcass decomposed at a slower rate than undisturbed buried carcasses. Carcasses placed above-ground are in direct contact with

certain abiotic factors, such as cold temperatures, wind and rainfall, and succession and colonisation by certain sets of arthropods can occur more easily. Abiotic factors are known to have a large impact on the rate of decomposition of a cadaver (Byrd & Castner 2001; George *et al*. 2013). The observations during this trial proved contrary to previous studies. Kelly (2006 *unpublished*), Kolver (2009 *unpublished*) and Hoffman (2014 *unpublished*) observed a faster rate of decomposition occurring during the winter season above-ground, compared to the present study, with the fresh stage lasting less than a week, and advanced decay being reached after 30 days in some cases. This was mainly due to an increased arthropod presence, particularly dipteran, during the winter months in these studies. In contrast to above-ground scenarios, buried cadavers are isolated from ambient temperatures, wind and rain, and are subjected to other sets of abiotic factors and biotic components driving decomposition processes. These alternative components include saprotrophs such as increased bacterial activity, fungal growth and differing sets of arthropod colonisation (Haglund & Sorg 1997; Tibbett & Carter 2008).

With decomposition occurring at a generally faster rate below-ground, biomass was seen to be lost at a faster rate on undisturbed buried carcasses, compared to the control carcass, on the majority of the excavation days (Figure 3.1.9). This shows that the general trend of decomposition and biomass loss was faster below-ground compared to above-ground. Although a generally faster rate of biomass loss was recorded on the buried carcasses, none of the carcasses had lost more than 20% of their biomass at the time of their respective excavations. This indicates that although decomposition and biomass loss was generally faster below-ground, the process was still relatively slow due to decreased arthropod succession and climatic conditions. Janaway (1996) reported on the decreased activity of microbial and soil organisms in very dry soils. The dry soils and cold climate during the trial period prevented increased microbial and soil organism activity, leading to a relatively slow decomposition rate and thus a low rate of biomass loss on buried carcasses.

Arthropod succession occurred at a much slower rate on the buried carcasses, compared to the control carcass, as first evidence of below-ground colonisation was recorded 21 days after burial by phorid species (Figure 3.1.14). Despite this slower successional rate, colonisation of the buried carcasses was seen to be more specific with only a few species occurring and on certain regions of the carcasses. Separate waves of succession were also noted with a further two dipteran species colonising buried carcasses 30 days after burial. This indicates differing time frames of succession occurring as the duration of burial increases. *Dermestes maculatus*

adults and larvae dominated over the majority of the trial period with fewer dipteran species occurring during the colonisation of the control carcass. Predation by this species became the main factor affecting dipteran succession. While successional studies by Kelly (2006 *unpublished*), Kolver (2009 *unpublished*) and Hoffman (2014 *unpublished*) recorded the occurrence of this species, it was only noted during the later stages of decomposition and was not in high abundance during active decay compared to the present study (Figure 3.1.10).

The results obtained indicate specific differences occurring between above- and below-ground arthropod succession and decomposition with the isolated environment of graves providing protection from climatic conditions, while further preventing mass colonisation by arthropod species.

SECTION 3.2:

A comparison of seasonality on above- and below-ground arthropod succession and decomposition: A summer trial

This section reports on the results obtained during the summer trial. Previous summer studies found decomposition and arthropod succession to be accelerated in terms of rate and abundance (Kelly 2006 *unpublished*; Gilbert 2014 *unpublished*). The main causative factors for such an increased rate corresponds to hot summer temperatures increasing bacterial and arthropod activity, as well as increased vegetation growth providing refugia (Byrd & Castner 2010). Variations in arthropod abundance are commonly associated with seasonal changes, giving rise to differences in faunal diversity and abundance within a region (Anderson 2010). Temperatures recorded during the trial are presented for both above- and below-ground situations and compared. Characteristics and groupings by Payne (1965) and Anderson & Laerhoven (1996) were used to record the stages of decomposition the summer carcasses experienced over the 60 day period. Arthropod succession was categorised according to presence and abundance while aggregation analyses were represented graphically. Statistical analyses are presented in terms of ordination as well as association and correlation between carcasses.

3.2.1 Ambient Temperatures, Grave Temperatures and Rainfall

Ambient temperatures during the summer trial showed fluctuations, with temperatures exceeding 30°C on a number of days. Comparisons of field temperatures at the study site with those recorded at the Universitas and Bram Fischer Airport weather stations generally indicated higher temperatures occurring at the study site (Figure 3.2.1). This can be attributed to the weather stations being located in built-up areas which caused the ambient temperatures to be lower due to buffering from elements, whereas the study site was open and exposed to high amounts of solar radiation and wind. Such differences illustrate how ambient temperature differs within the Bloemfontein area. This confirms the practice of adjusting temperature accordingly at a crime scene during investigations.

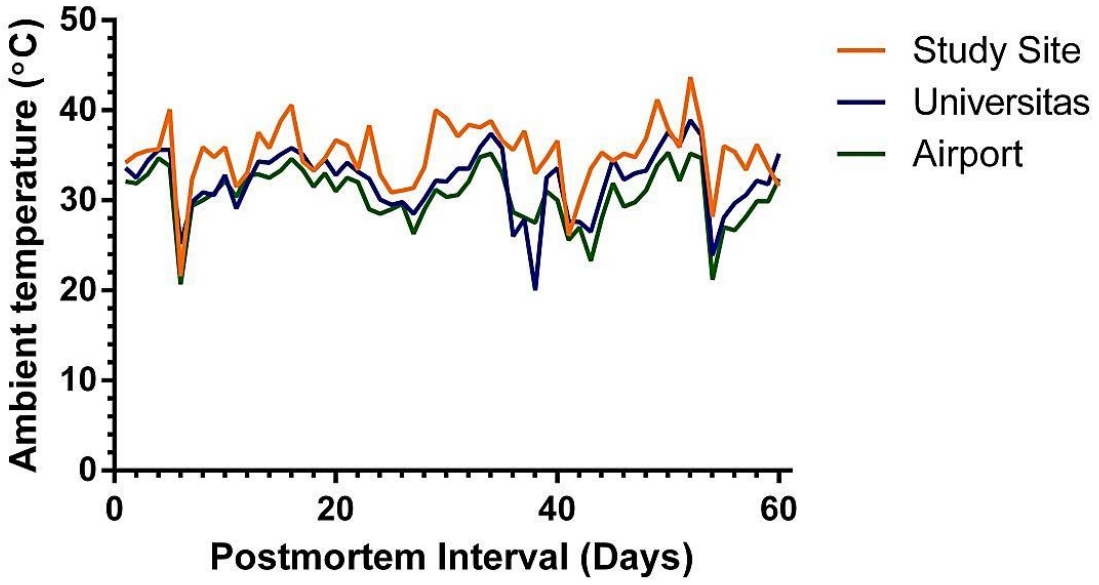

Figure 3.2.1: Midday ambient temperatures recorded at the study site and the Universitas and Bram Fischer Airport weather stations during the summer trial in 2014/2015.

Summer rainfall reached a total of 140 mm over the 60 day period (Figure 3.2.2). Heavy rainfall caused large decreases in ambient temperatures. On day 6 of the trial a total of 44 mm was recorded and caused many of the graves to flood and become impacted (Figure 3.2.3). All of the graves contained clay based soils in differing concentrations which prevented adequate drainage after rainfall, and thus remained waterlogged for extended periods of time.

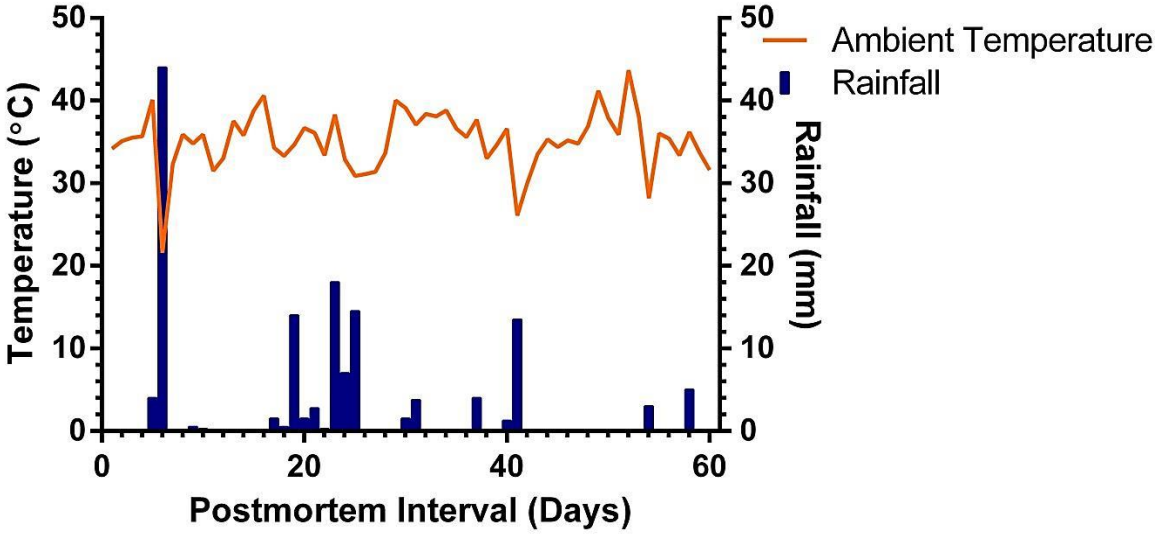

Figure 3.2.2: Rainfall and midday ambient temperatures at the study site during the summer trial in 2014/2015.

Figure 3.2.3: Impact of rainfall on summer graves, causing flooding and impaction of soil on grave six (day 12, 02/01/2015).

During summer the internal grave temperatures of the disturbed grave one were in the range of between 20ºC to 30ºC (Figure 3.2.4). Surface temperatures recorded showed large fluctuations, corresponding with rainfall, and a number of surface readings exceeded 40ºC. The internal grave temperatures of the disturbed grave one overlapped extensively during the trial period and rarely deviated from one another. At certain times ambient and surface temperatures were seen to drop below internal grave temperatures during periods of heavy rainfall on days 6 and 54 respectively. Despite the impact of rainfall, internal grave temperatures remained within the 20ºC to 30ºC range, indicating that the grave provided a fairly stable environment and illustrates the impact of burial in regards to the isolation of carcasses from above-ground abiotic factors. A drop in the temperature of the grave carcass was seen after heavy rainfall on day 6 (Figure 3.2.4). During this time the soil of the grave became extremely waterlogged and compaction was seen around the body. The large amount of water in the grave cooled the carcass until a sufficient amount had drained away, after which the temperature of the carcass increased.

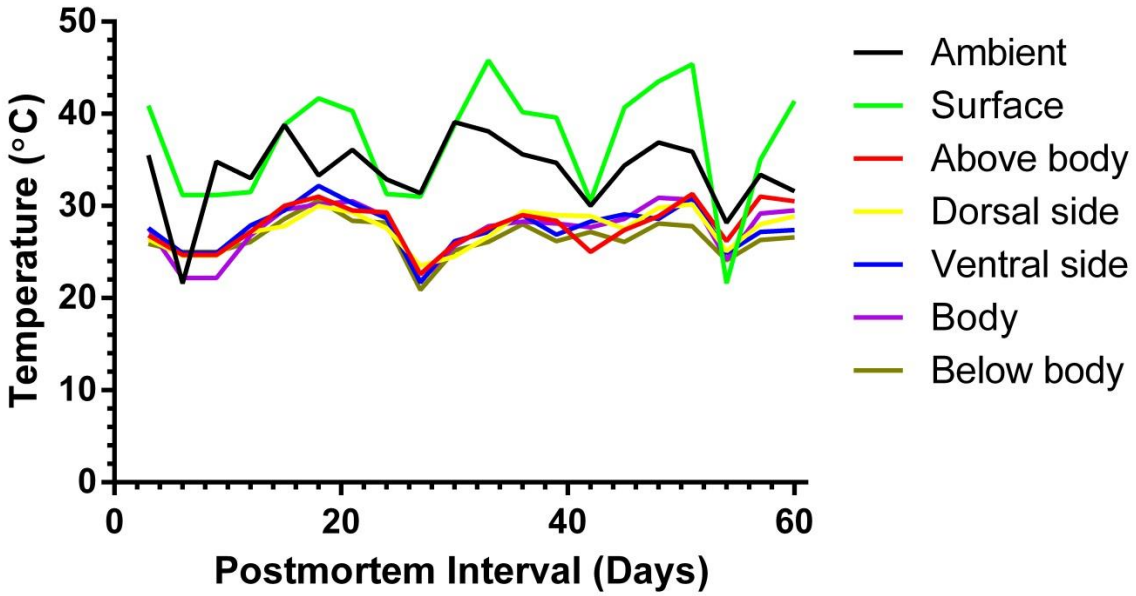

Figure 3.2.4: Grave temperatures of the disturbed grave one in summer (taken every third day), mapped with midday ambient temperatures as recorded during the 2014/2015 summer trial.

The temperature of the control carcass was taken every third day of the trial period, coinciding with the excavations of the disturbed grave one carcass (Figure 3.2.5). Noticeable differences in temperature were seen between the control and disturbed grave one carcass. Generally, a higher body temperature was recorded on the control carcass compared to the disturbed grave one carcass. Exceptions did occur on day 6 and 54 of the trial, however, when heavy rainfall caused the control carcass temperature to drop below, or to the level of, that of the disturbed grave one carcass. This illustrates the degree to which an above-ground carcass is affected by environmental conditions. The fluctuations of the control carcass temperatures corresponded to fluctuations in ambient temperature and rainfall during the trial. Solar radiation increased the temperature of the control carcass to temperatures exceeding ambient temperatures, with the exception of day 6 and 54. In comparison to the disturbed grave one carcass, the control carcass showed more temperature fluctuations due to it being completely exposed to weather conditions throughout the trial period.

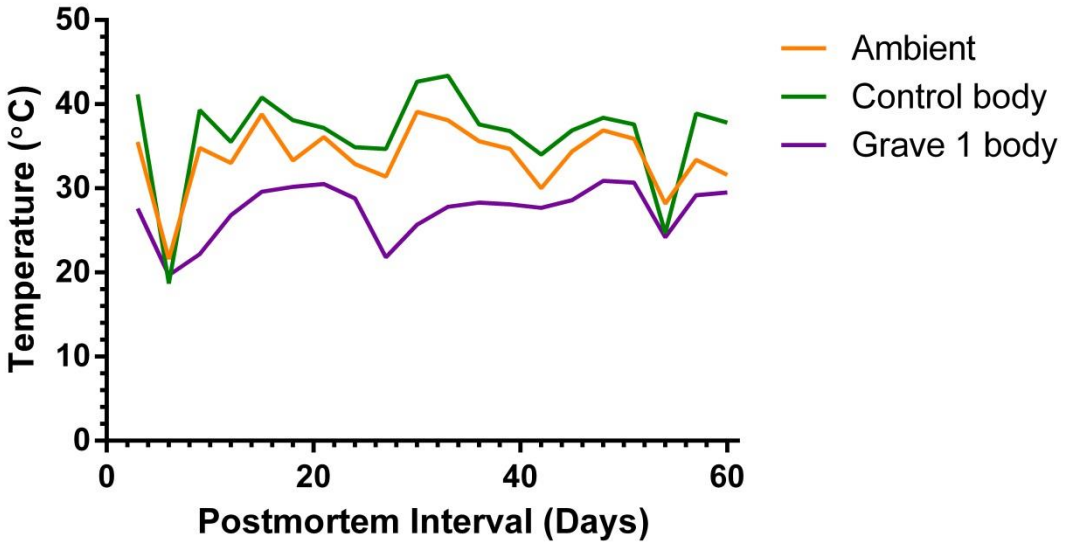

Figure 3.2.5: Temperatures recorded every third day from the control carcass and the disturbed grave one carcass during the 2014/2015 summer trial, mapped with summer ambient temperatures.

Temperatures recorded from the undisturbed graves during excavations showed major differences between surface and internal grave temperatures (Figure 3.2.6). Surfaces of the graves were exposed to high amounts of solar radiation over extended periods of time during the summer trial. This is evident in the high temperatures recorded on the surface of each grave at the time of excavation. Abnormalities were seen, however, during the excavation of grave two (day 7) in which the surface temperature was lower than the internal grave temperatures due to the influence of rainfall. As depth increased, decreases in temperature were recorded compared to surface temperatures. The range of the internal grave temperatures was between 24^0C to 34^0C. This temperature range was similar to that experienced in the disturbed grave one. Despite increased rainfall and ambient temperatures above-ground, internal grave temperatures remained fairly constant within the excavated graves. Slight variations in internal grave temperatures were seen on the undisturbed graves during the trial, with the lowest internal grave temperatures recorded occurring in grave two (day 7) and five (day 30). Carter & Tibbett (2006) reported that the occurrence of heavy rainfall can cause clay soil to compact around grave carcasses and reduce air circulation within the grave. This in turn can affect internal grave temperatures depending on moisture levels. The slight variations in internal grave temperatures experienced during the summer trial, due to heavy rainfall, illustrates that certain above-ground environmental conditions can affect buried carcasses if in sufficient quantities and depending on the depth of burial.

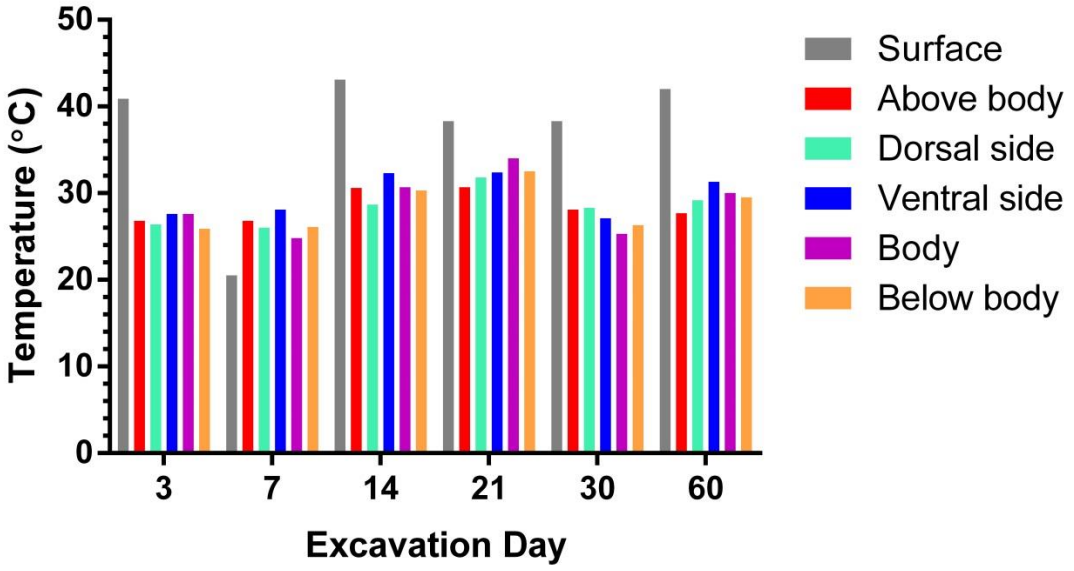

Figure 3.2.6: Grave temperatures of undisturbed graves, recorded during excavations, during the 2014/2015 summer trial.

3.2.2 Carcass Decomposition

A total of five stages of decomposition were observed during the 60 day summer trial between the above- and below-ground carcasses (Table 3.2.1).

1. *Fresh Stage*

   Once placed in the field (day 0) the control carcass remained in the fresh stage for less than 24 hours, entering transition to bloat by day 1 (Figure 3.2.8). High temperatures at this stage were the major contributing factors to such a fast transition. According to Carter *et al*. (2008), this provided a beneficial environment for bacterial activity to take place. Below-ground, the fresh stage lasted until day 3 in grave one, indicating a slower rate of decomposition. Large numbers of arthropods, particularly Diptera, were recorded on the control carcass from day 0 onwards while no such activity was recorded below-ground.

2. *Bloated Stage*

   The onset of bloat occurred quickly above-ground with the control carcass inflating drastically. However, this stage did not last as long as the subsequent stages of decomposition, and by day 4 the carcass was entering the transition stage to active decay. Arthropod presence increased with large numbers of dipteran larvae feeding on the control carcass. No bloated stage was recorded below-ground, and by day 6 and 7 graves one and

two respectively had already entered active decay (Figure 3.2.8). Such a rapid rate of decomposition can be attributed to heavy rainfall experienced on days 5 and 6 of the study. The soil of all graves became waterlogged and compacted, effectively sealing off many of the graves. Carter *et al*. (2010) found that the presence of a moist, isolated environment can provide adequate circumstances for both anaerobic and aerobic bacteria to function. This, in turn, may have increased the rate of decomposition below-ground along with soil compaction accelerating deflation of the buried carcasses.

3. *Active Decay*

High ambient temperatures and rainfall ensured a fast transition to active decay above-ground during the summer trial. The control carcass remained in active decay from day 5 until day 10 (Figure 3.2.8). Dipteran species dominated the control carcass with a high number of larvae colonising the carcass.

It was estimated that the below-ground carcasses entered active decay at the same time as the control carcass, as the carcasses in graves one and two were recorded as being in active decay on days 6 and 7 respectively during excavations. It was estimated that the remaining undisturbed grave carcasses remained in active decay until day 20, while the disturbed grave one carcass remained in active decay for a longer period of time from day 6 until day 29 (Figure 3.2.8).

Of interesting note was the carcass in grave six (day 60), which, despite being undisturbed for 60 days, was found to be in active decay while the previously excavated grave four (day 21) and five (day 30) carcasses had already entered advanced decay (Figure 3.2.8). The mainly clay soil composition of grave six is a possible contributing factor as to the slower rate of decomposition experienced by the carcass. All other graves contained a soil mixture of loam and clay which allowed drainage of water to occur at a faster rate, whereas the mainly clay soil of grave six caused the grave to remain waterlogged for an extended period of time, and drainage did not occur at such a fast rate. This shows the impact of heavy rainfall and soil composition on buried cadavers, and the effect this can have on PBI determination when based upon the stage of decomposition.

Table 3.2.1: Summarisation of decomposition stages experienced during the 2014/2015 summer trial for both the above- and below-ground carcasses, as well as most dominant arthropods present during these stages.

Stage of decomposition	Arthropods present
FRESH • Above-ground: Day 0 to 1 • Below-ground: Day 0 to 3 • No odour present • Stage persisted for less than one day above-ground • Discolouration of skin on neck, belly, arms and torso of control carcass	**Diptera** Calliphoridae: *Lucilia* spp. (adults) (control only) *Chrysomya marginalis* (adults) (control only) *Chrysomya albiceps* (adults) (control only) *Chrysomya chloropyga* (adults) (control only) Muscidae: *Musca domestica* (adults) (control only) **Hymenoptera** Formicidae: *Anoplolepis custodiens* (control only)
BLOAT • Above-ground : Day 2 to 3 (Transition, day 1) • Below-ground: Unknown, likely occurred between day 3 and 6 • Stage characterised by inflation of body caused by build-up of gases within carcass due to microbial activity • No bloated stage seen on buried carcasses • Bodily fluids seen to leak out of body through major orifices such as mouth, anus and ears on control carcass • Oviposition of several dipteran species noted during this stage as well as high maggot activity on control carcass	**Diptera** Calliphoridae: *Lucilia* spp. (adults and maggots) (control only) *Chrysomya marginalis* (adults and maggots) (control only) *Chrysomya albiceps* (adults and maggots) (control only) *Chrysomya chloropyga* (adults and maggots) (control only) Muscidae: *Musca domestica* (adults) (control only) **Hymenoptera** Formicidae: *Anoplolepis custodiens* (control only) *Linepithema* sp. (control only)
ACTIVE DECAY • Above-ground: Day 5 to 10 (Transition, day 4) • Below-ground: Day 6 to 27 (Transition, day 4 to 5) • Strong odour of decay present from all carcasses • Large amount of decomposition liquids present beneath all carcasses • Large loss of biomass on control carcass during stage due to feeding by dipteran maggots • Body tissue removed from control carcass due to feeding by dipteran maggots • Severe skin slippage and adipocere formation on buried carcasses due to flooding of graves • Most dominant arthropods noted to be dipteran species on control carcass • Mites most dominant arthropod species on buried carcasses • High rate of competition between arthropod species on control carcass seen with predation by ant species on dipteran maggots and newly emerged adults • Maggots not seen to migrate in large numbers from control carcass during decomposition • Less arthropod species present on buried carcasses compared to control carcass	**Diptera** Calliphoridae: *Lucilia* spp. (adults and maggots) (control only) *Chrysomya marginalis* (adults and maggots) (control only) *Chrysomya albiceps* (adults and maggots) (control only) *Chrysomya chloropyga* (adults and maggots) (control only) Muscidae: *Musca domestica* (adults) (control only) Sarcophagidae: *Sarcophaga* sp. (maggots and pupae) (control & graves) Phoridae: *Megaselia scalaris* (adults) (control & graves) **Coleoptera** Dermestidae: *Dermestes maculatus* (adults) (control only) Cleridae: *Necrobia rufipes* (adults) (control only) Silphidae: *Thanatopilus mutilatus* (adults) (control only) Staphylinidae: *Aleochara* sp. (adults and larvae) (graves only) **Hymenoptera** Formicidae: *Anoplolepis custodiens* (control only) *Linepithema* sp. (control only) **Acari** Acaridae: *Sancassania mycophagus* (graves only)

Table 3.2.1: Continued.

Stage of decomposition	Arthropods present
ADVANCED DECAY • Above-ground: Day 12 to 38 (Transition, day 11) • Below-ground: Day 21 to 60 • Little biomass remaining on control carcass • Weak odour of decay present • Skin remained covering body and dried out slowly on control carcass as time progressed • Buried carcasses remained moist due to flooding • Adipocere formation still present on buried carcasses • Large numbers of newly emerged dipteran species seen during first week of stage on control carcass • Coleoptera species became dominant group on control carcass as stage progressed • Few arthropod species present on buried carcasses during this stage	**Diptera** Calliphoridae: *Lucilia* spp. (adults) (control only) *Chrysomya marginalis* (adults) (control only) *Chrysomya albiceps* (adults) (control only) *Chrysomya chloropyga* (adults) (control only) Muscidae: *Muscina stabulans* (adults) (control only) *Musca domestica* (adults) (control only) Sarcophagidae: *Sarcophaga* sp. (adults and maggots) (control & graves) Phoridae: *Megaselia scalaris* (adults) (graves only) **Coleoptera** Dermestidae: *Dermestes maculatus* (adults and larvae) (control only) Cleridae: *Necrobia rufipes* (adults) (control only) Staphylinidae: *Aleochara* sp. (adults and larvae) (graves only) **Hymenoptera** Formicidae: *Anoplolepis custodiens* (control only) *Linepithema* sp. (control only) **Acari** Acaridae: *Sancassania mycophagus* (graves only)
DRY REMAINS • Above-ground: Day 40 to 60 (Transition, day 39) • Below-ground: Not reached • Control carcass completely dried out, only a few patches of hair and skin remained • Skeleton of control carcass fully exposed • Coleoptera species dominant group	**Coleoptera** Dermestidae: *Dermestes maculatus* (adults and larvae) (control only) Cleridae: *Necrobia rufipes* (adults) (control only) **Hymenoptera** Formicidae: *Anoplolepis custodiens* (control only) *Linepithema* sp. (control only)

4. *Advanced Decay*

Advanced decay dominated the decomposition time frame for all carcasses, with the control carcass being the first to enter advanced decay on day 12 and remained in this stage until day 39 (Figure 3.2.8). This was followed by the carcass in the undisturbed grave four on day 21, while the disturbed grave one carcass was the last to reach the advanced stage of decay on day 30 of the trial. While it can be estimated that the undisturbed grave two (day 7) to five (day 30) carcasses remained in advanced decay until the end of the trial, the grave six carcass was still in active decay after 60 days (Figure 3.2.8). The disturbed grave one carcass also remained in advanced decay until the end of the trial due to high levels of disturbance caused by excavations (Figure 3.2.8).

During this stage, the control carcass became dry and leathery as fluids continued to escape from the body. Rain during this time did not rehydrate the carcass since rainfall was followed by high ambient temperatures which increased the rate of evaporation around the carcass. This drying of the carcass attracted coleopteran species. The buried carcasses, however, remained moist during advanced decay due to the graves becoming waterlogged. High levels of moisture in the soil provided an environment which was conducive of skin slippage and adipocere formation (Figure 3.2.7). Such an occurrence was seen in previous studies by Fiedler & Graw (2003) and Forbes *et al*. (2005a). Fungal growth was minimal on the buried carcasses due to waterlogging preventing adequate oxygen levels. Adequate oxygen is a prerequisite for fungal growth (Hofkin 2010).

Figure 3.2.7: Occurrence of skin slippage and adipocere formation on the undisturbed grave five carcass (day 30, 20/01/2015) during the summer trial.

5. *Dry Remains*

Only the control carcass reached the dry remains stage by the end of the trial period. The control carcass entered the dry remains stage on day 40 and remained in this state until the end of the trial (Figure 3.2.8). Coleopteran species continued to dominate the control carcass until the end of the trial with *Dermestes maculatus* adults and larvae feeding on the remaining dry tissue.

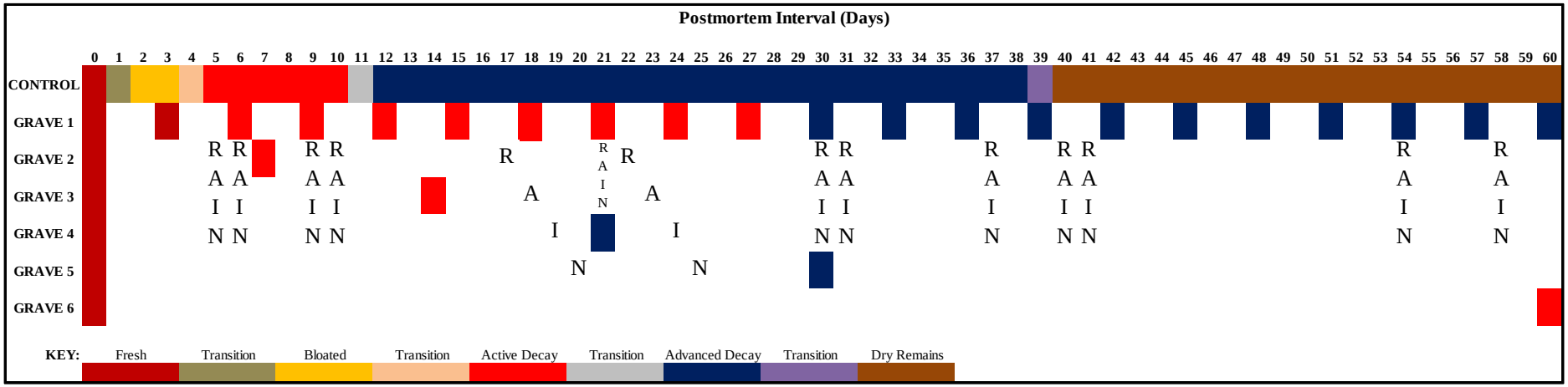

Figure 3.2.8: Stages of decomposition experienced by the carcasses during the 2014/2015 summer trial.

3.2.3 Biomass Loss

Summer carcasses experienced a fast rate of biomass loss over the trial period (Figure 3.2.9). The control carcass lost more than 80% of its biomass by the end of the trial period. With the control carcass being exposed to more biotic and abiotic factors, as well as increased rainfall having an impact on decomposition (Archer 2004), the control carcass was able to decompose at a fast rate. The undisturbed grave carcasses lost less biomass, compared to the control carcass, with none losing more than 50% of their biomass at the times of excavation. The carcass in grave six (day 60) was seen to lose even less biomass than the other graves. With the soil of grave six consisting mainly of clay, and heavy rainfall occurring periodically during the trial, the grave became waterlogged, slowing decomposition and decreasing subsequent biomass loss.

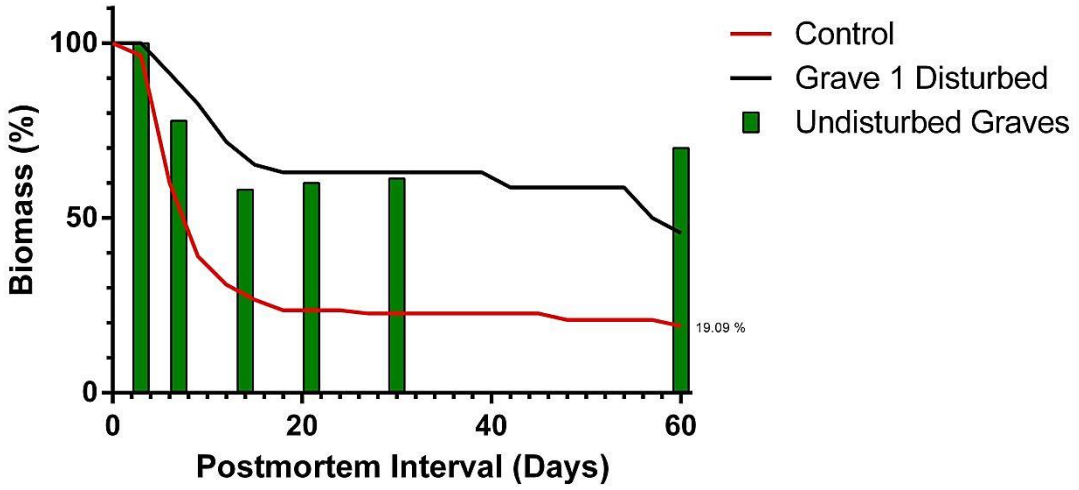

Figure 3.2.9: Rate of biomass loss from carcasses during the 2014/2015 summer trial.

The rate of biomass loss recorded from the carcass in the disturbed grave one showed periods of no decrease in biomass. The presence of fewer arthropods on the carcass, along with heavy rainfall, contributed to the slower rate in biomass loss compared to the control carcass.

Despite heavy rainfall and reduced arthropod activity, all buried carcasses underwent a fast rate of biomass loss. This can be attributed to the temperature of the graves being in a range supportive of bacterial and enzymatic activity; factors which drive decomposition and, subsequently, biomass loss (Schimel & Bennett 2004; Pechal *et al*. 2013).

3.2.4 Arthropod Succession

Samples taken from each grave before burial of the carcasses showed a high species diversity present below-ground (Table 3.2.2). The most common species belonged to the family Formicidae. Occurrences of coleopteran species were found in graves one, three and five, as well as a single centipede *Cormocephalus* species in grave four. Vegetation abundance and more favourable conditions within the field account for this increase in species diversity due to increased temperatures and presence of suitable food and refugia.

Table 3.2.2: Arthropod species present in the 2014/2015 summer graves before burial of the carcasses, sampled on 20/12/2014.

Arthropods present	Grave 1	Grave 2	Grave 3	Grave 4	Grave 5	Grave 6
Coleoptera						
Apionidae:						
Cylas sp.	-	-	**X**	-	-	-
Scarabaeidae:						
Scarabaeidae sp. larva	**X**	-	-	-	**X**	-
Hymenoptera						
Formicidae:						
Anoplolepis custodiens	**X**	**X**	**X**	**X**	-	**X**
Camponotus maculatus	-	-	-	-	**X**	-
Camponotus sp.	-	-	-	**X**	-	-
Linepithema sp.	-	-	**X**	**X**	**X**	-
Messor capensis	-	**X**	**X**	-	**X**	-
Solenopsis punctaticeps	-	**X**	-	-	**X**	-
Scolopendromorpha						
Scolopendridae:						
Cormocephalus sp.	-	-	-	**X**	-	-

Differences between above- and below-ground arthropod succession was noted as the summer trial progressed.

1. *Above-ground*

   Arthropod succession occurred at a fast rate during the summer trial period, with higher species diversity and abundance occurring above-ground compared to below-ground. Adult dipteran species arrived on the control carcass minutes after placement in the field. The calliphorid species *Chrysomya marginalis* (Wiedemann), *Chrysomya albiceps*, *Chrysomya chloropyga* and *Lucilia* species were seen on the control carcass by day 1,

along with a few *Musca domestica* Linnaeus individuals (Figure 3.2.10). Large numbers of dipteran larvae were seen feeding on the control carcass by the second day of the trial, indicating oviposition had taken place immediately after the carcass was placed in the field. The majority of these larvae were identified as *C. marginalis*, *C. albiceps* and *C. chloropyga*. Maggot numbers remained high during the first week, after which abundance dropped as the immatures pupated. Dipteran adult abundance remained relatively high during this time, fluctuating as time progressed.

From day 14 onwards, adult dipteran abundance increased to higher numbers than during initial colonisation. Many of the adults were recorded as being newly emerged (Figure 3.2.11), indicating that dipteran larvae on the control carcass did not migrate away from the body before pupation, leading to an influx of dipteran abundance in the later stages of decomposition. Heavy rainfall is the most likely explanation for the larvae not migrating from the body. A carcass provides suitable shelter for pupae as rainfall occurs, and can act as a temperature regulator as high ambient temperatures can prove fatal to certain dipteran larvae (Voss *et al*. 2014). After the emergence event, dipteran abundance gradually decreased up to the point where only a few adult individuals were seen at random intervals belonging to families other than Calliphoridae. No calliphorids were recorded from day 24 onwards (Figure 3.2.10).

Other Diptera (muscids and piophilids) were effectively outcompeted by calliphorids as these species were not identified among the immatures colonising the control carcass. Between 10 and 50 *M. domestica* adults were recorded on the body during the first three weeks of the trial. This species is largely known to feed upon decomposing carcasses but can easily be outcompeted (Kneidel 1984). However, it is considered having potential as a forensic indicator as it has been found on cadavers in large quantities when calliphorids failed to oviposit (Chong Chin *et al*. 2008; Horenstein *et al*. 2010).

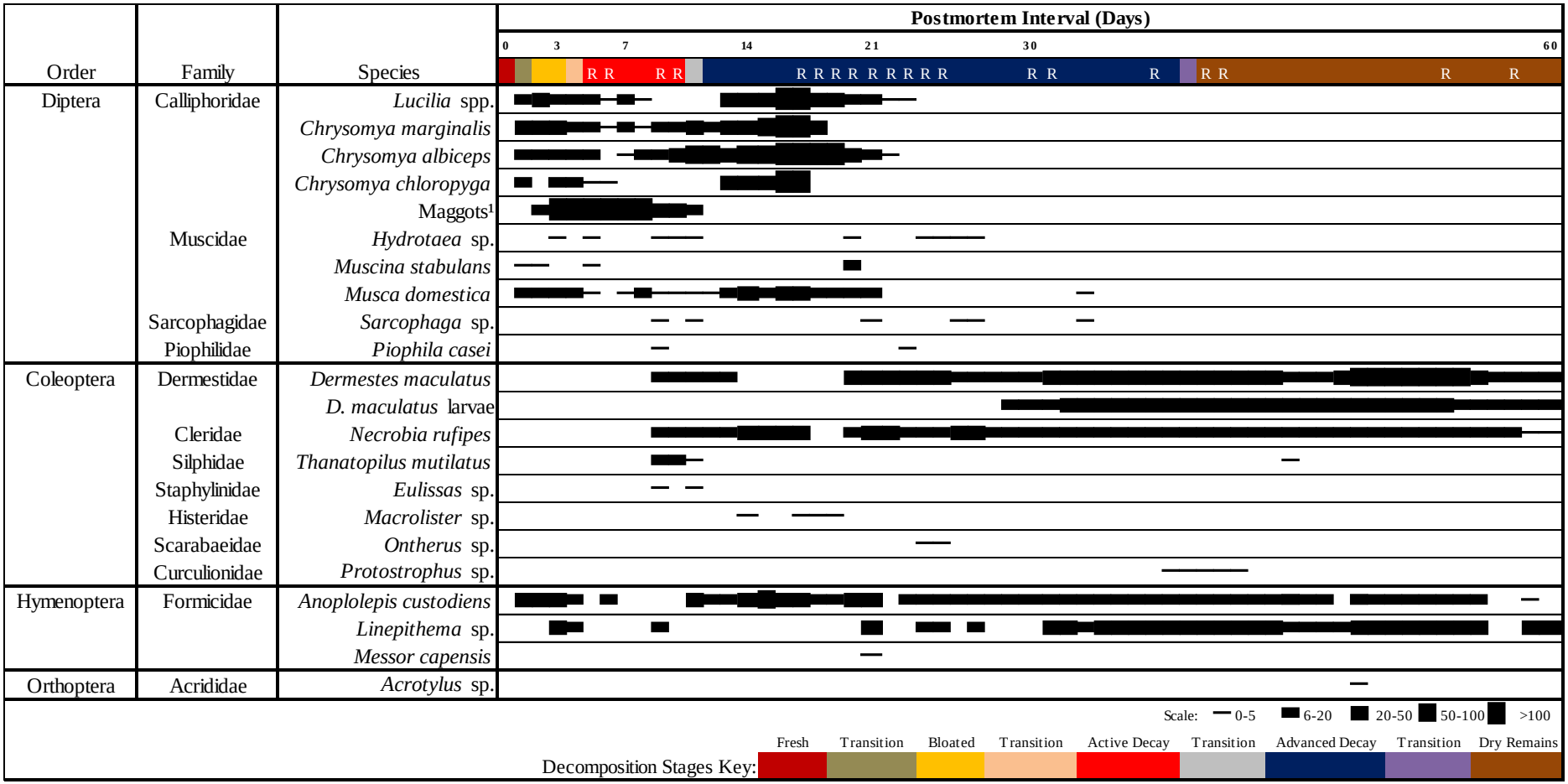

Figure 3.2.10: Arthropod succession on the control carcass during the 2014/2015 summer trial. Maggots[1] – only Calliphoridae species. (R – Rainfall).

Figure 3.2.11: Newly emerged Calliphoridae adults, lacking colouration and fully developed wings, on the 2014/2015 summer control carcass (day 17, 07/01/2015).

During the last few days of active decay (day 9 to 11) coleopteran species were seen colonising the control carcass, the most abundant species being *D. maculatus* and *Necrobia rufipes* adults. At the time of the newly emerged dipteran adults occurring, coleopteran abundance of *D. maculatus* individuals dropped dramatically for a few days before picking up again (Figure 3.2.10). From day 20 onwards, *D. maculatus* and *N. rufipes* adults remained on the control carcass until the end of the trial. Larvae of *D. maculatus* were sampled during the advanced decay stage of the control carcass, but no immatures of *N. rufipes* were found.

Large numbers of Formicidae species were seen on and around the control carcass with *Anoplolepis custodiens* Smith being the most abundant. Ant species are often considered as a factor which can affect PMI determination due to their predatory behaviour on dipteran immatures (Campobasso *et al*. 2009). While predation by ants was seen on the control carcass during initial colonisation, the high abundance of dipteran immatures prevented any noticeable effect on succession. High rainfall was also seen to prevent ants from colonising the body due to run off of water from the carcass and the area surrounding it. Increased predation by ants on dipteran adults was noted during the adult emergence

event (Figure 3.2.12). Due to the newly emerged adults not being as mobile as more mature individuals they were more vulnerable to predation.

Figure 3.2.12: Predation by ants (*Anoplolepis custodiens*) on a newly emerged Calliphoridae adult during the 2014/2015 summer trial (day 20, 10/01/2015).

2. *Below-ground*

Only four arthropod species were found during excavations (Figure 3.2.13). The first records of arthropod species present on the undisturbed grave carcasses was on day 21, indicating that colonisation may have occurred from day 14 onwards. Adult *Megaselia scalaris* individuals were sampled from grave four along with adults and larvae of a staphylinid *Aleochara* Gravenhorst species. Abundance of the dipteran species was low and no immatures were seen on the body. During the excavation on day 30, no arthropod species were sampled from the grave five carcass (day 30), despite it being undisturbed for longer compared to the previous excavated graves. This can be attributed to the soil composition of this grave being of a high clay-loam mixture both on the surface and at the bottom of the grave, effectively sealing the grave during heavy rainfall and preventing colonisation by arthropods. The carcass of grave six (day 60) yielded *M. scalaris* adults

along with open sarcophagid pupae in the soil above the carcass. Staphylinid *Aleochara* sp. adults and larvae, and large numbers of the astigmatid mite *Sancassania mycophagus* (Mégnin), as identified by Dr Louise Coetzee and Dr Edward Ueckermann, also occurred.

Despite increased abundance of *M. scalaris* adults in grave six (day 60), no immature stages of this species were found on the carcass. The mite species *S. mycophagus* colonised the buried carcass of grave six (day 60) extensively in easily noticeable clusters (Figure 3.2.14). Adults of the staphylinid *Aleochara* sp. were seen to prey extensively on these mites and adult *M. scalaris* individuals, while their larvae were seen roaming on the body (Figure 3.2.15). This is indicative of a possible ecological interaction between the three species. The exact effect feeding of this mite species has on decomposition of buried cadavers, its interaction with other species, and possible effect on PBI has yet to be established. The predatory behaviour of the staphylinid *Aleochara* sp. indicates that Coleoptera may impact below-ground arthropod succession, and this in turn may affect PBI estimations.

Although having a slower rate of decomposition in comparison to the undisturbed graves, the disturbed grave one carcass provided additional insight into the rate of arthropod succession below-ground during summer. Only three of the species found in the undisturbed graves were found during the excavations of grave one, with a single sarcophagid larva sampled on day 18 (Figure 3.2.16). The presence of this immature in the disturbed grave one correlates with the inference that colonisation may have started from day 14 onwards in the undisturbed graves. Rainfall and flooding of the grave may have been the cause for only a single sarcophagid larva being sampled, as further oviposition and development was prevented. Colonisation occurred again from day 18 to 36 as more sarcophagid larvae were sampled from day 36 to 42 (Figure 3.2.16). Colonisation by the mite *S. mycophagus* and staphylinid *Aleochara* sp. occurred simultaneously from day 36 to 39. It is thought that the presence of *S. mycophagus* lead to the arrival of *Aleochara* sp. individuals, similar to the simultaneous occurrence of *M. scalaris* and *Aleochara* sp. individuals in the undisturbed grave four (day 21), as species of this genus are known scavengers and predators (Anton *et al*. 2011), further indicating a possible ecological relationship between these three species.

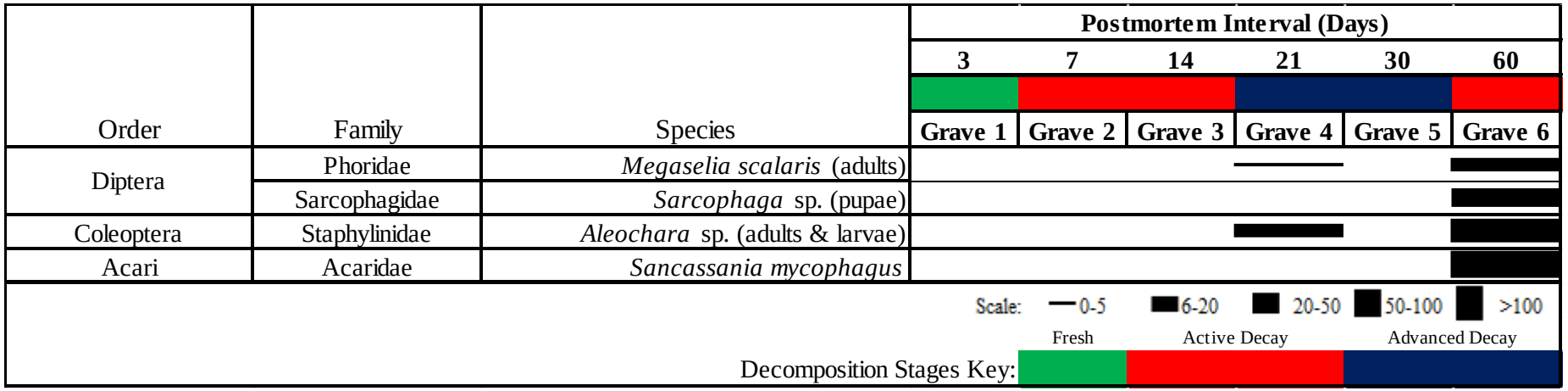

Figure 3.2.13: Arthropod succession on the undisturbed buried carcasses during the 2014/2015 summer trial.

Figure 3.2.14: Clusters of the mite species *Sancassania mycophagus* present on the grave six carcass during the 2014/2015 summer trial (day 60, 19/02/2015).

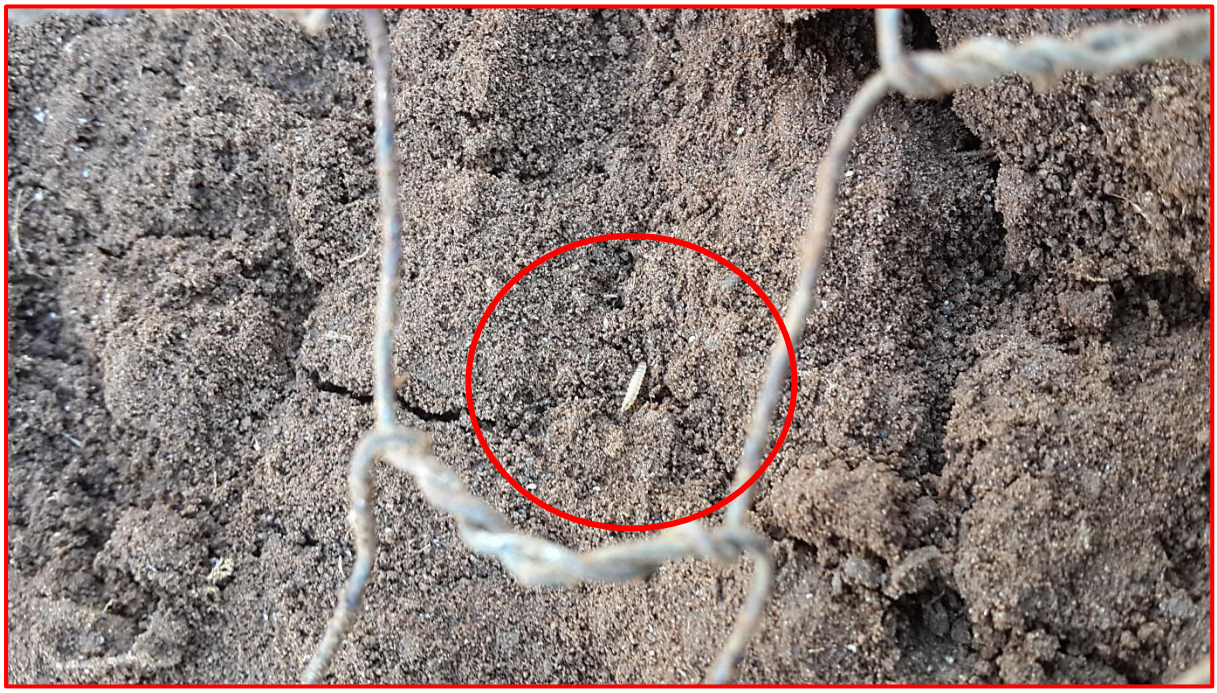

Figure 3.2.15: Staphylinid *Aleochara* species larva roaming on the buried carcass of grave six (day 60, 19/02/2015) during the 2014/2015 summer trial.

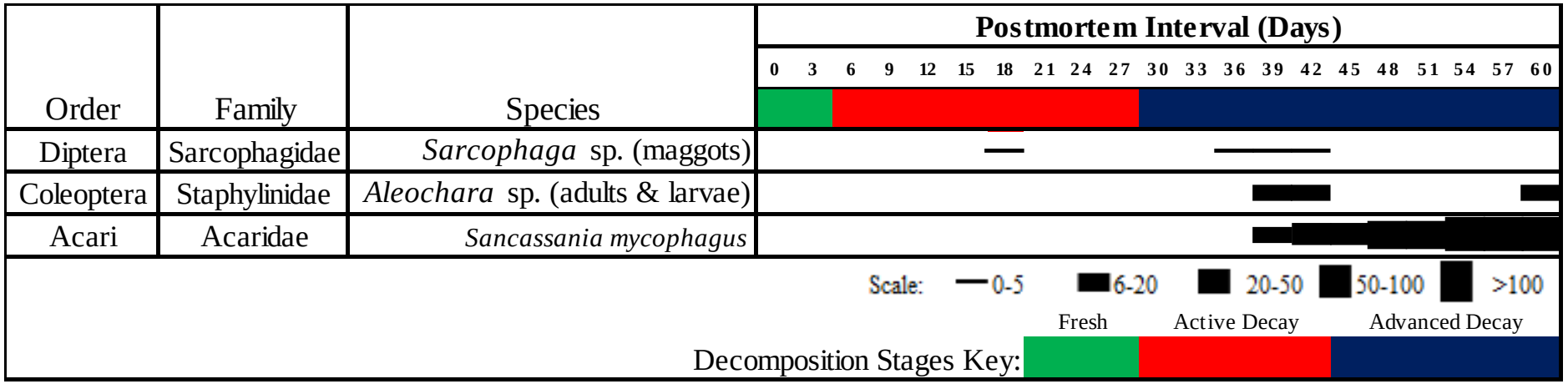

Figure 3.2.16: Arthropod succession on the disturbed grave one carcass during the 2014/2015 summer trial.

3.2.5 Arthropod Aggregation Sites

Arthropod distribution was recorded daily on the control carcass (Figure 3.2.17) and during each excavation (Figure 3.2.18) for the summer trial. Differences in distribution differed between the control and colonised grave carcasses.

A number of dipteran oviposition sites were noted on the control carcass during the first few days of the summer trial. Nearly all of these sites yielded larvae which covered the entirety of the body (Figure 3.2.17). The majority of these larvae consisted of Calliphoridae species which outcompeted other dipteran species. While Diptera dominated the top and sides of the control carcass during the early stages of decomposition (day 1 to 21), coleopteran species dominated the underside of the body during the later stages (day 22 to 60). The majority of these species consisted of *D. maculatus* and Histeridae species adults. Certain coleopteran species were seen to feed on the eggs oviposited by dipteran species. Larvae of *D. maculatus* sampled during later decomposition stages (day 28 to 60) occurred mainly beneath the body. After rainfall, and subsequent vegetation growth, ants were seen to roam in the vicinity of the control carcass and preyed on dipteran adults and larvae. These ant species were seen to occur mainly beneath the body and around the legs and sides of the carcass. Extensive overlaps in species distribution can be seen occurring on the control carcass (Figure 3.2.17).

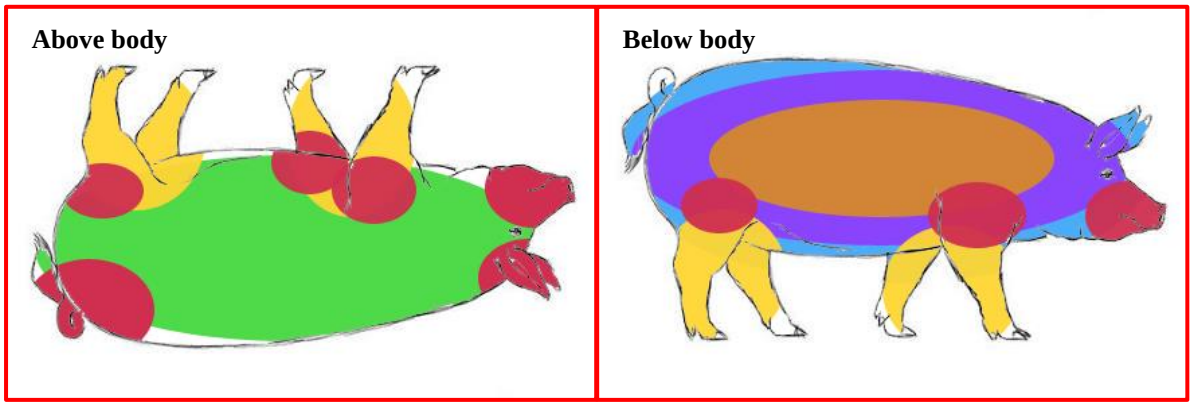

Figure 3.2.17: Arthropod distribution on the 2014/2015 summer control carcass. Red: Emerged dipteran oviposition sites; Green: Distribution of dipteran immatures; Blue: *Dermestes maculatus* distribution; Orange: Distribution of *Dermestes maculatus* immatures; Purple: Distribution of remaining coleopteran species; Yellow: Formicidae distribution.

Species distribution on colonised summer buried carcasses, in both disturbed and undisturbed graves, showed specific regions being colonised with overlaps in distribution occurring

(Figure 3.2.18). Dipteran species were less prominent on the buried carcasses, while coleopteran species and a single mite species dominated.

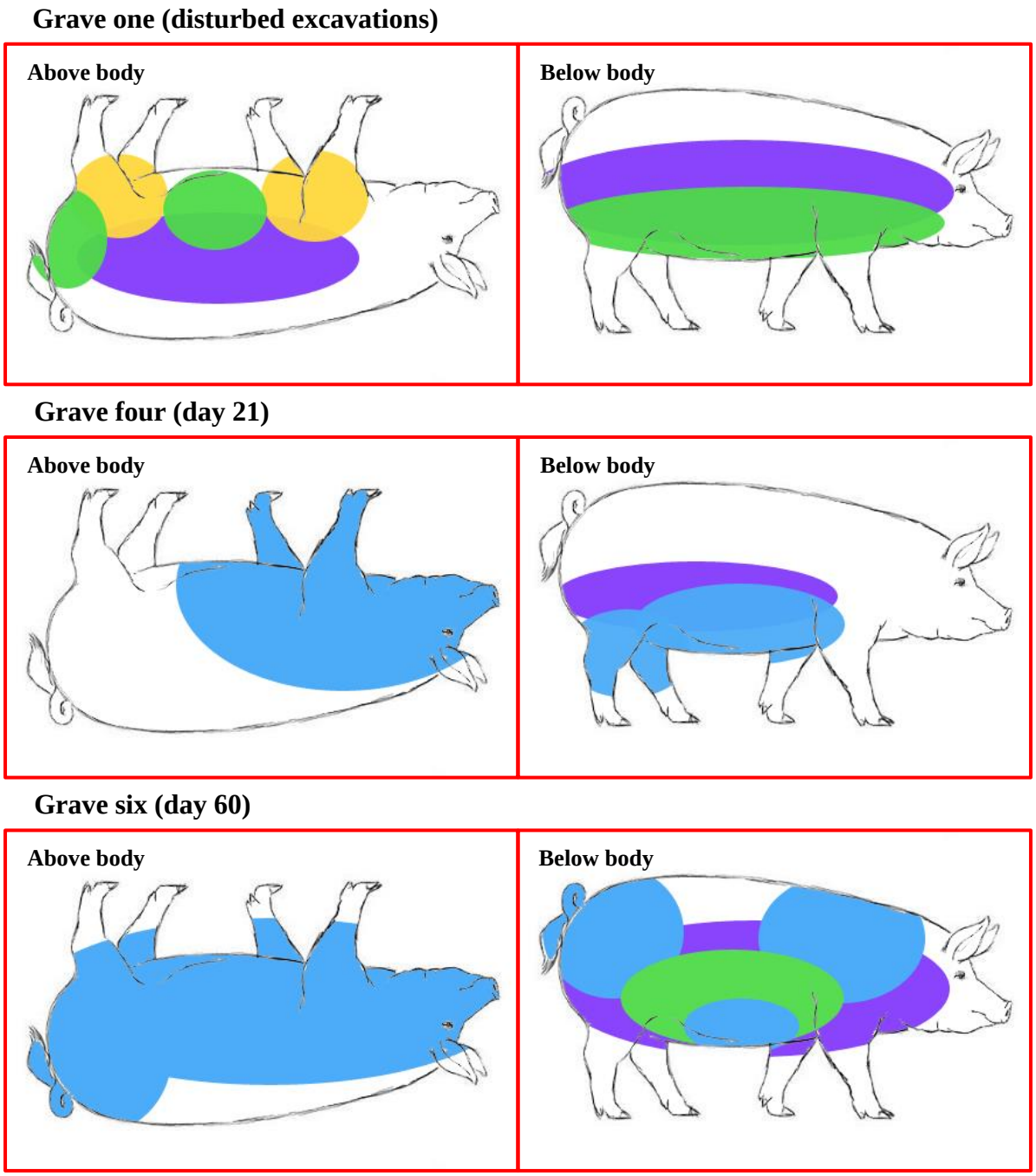

Figure 3.2.18: Arthropod distribution on the colonised summer grave carcasses during 2014/2015. Blue: Distribution of Phoridae adults; Yellow: Distribution of *Sarcophaga* species immatures; Purple: Distribution of *Aleochara* adults and larvae; Green: Distribution of mites (*Sancassania mycophagus*).

The only dipteran specimens sampled from the grave six carcass were Phoridae adults, and only the species *M. scalaris* was found. Phoridae adults colonised specific regions on the

body, mainly on the legs, hooves and stomach. While sarcophagid pupae were sampled from grave six (day 60), they occurred within the soil above the body and not on the carcass itself, therefore, they are not included in the aggregation analysis. Overlaps between the Phoridae adults and the staphylinid *Aleochara* sp. were noted on the undisturbed grave four (day 21) and six (day 60) carcasses. Many of the staphylinid adults were seen preying on the dipteran adults. The mite species *S. mycophagus* was in high abundance on the disturbed grave one carcass and the undisturbed grave six carcass (day 60), with the main colonisation site being on the stomach. Distribution overlaps between the mite species and the staphylinid *Aleochara* sp. were seen to occur extensively on these carcasses, and predation by the staphylinids on the mites was observed.

3.2.6 Statistical Analysis

Correspondence analysis ordination for summer showed typical clustering of the majority of sampled species around the control carcass (Figure 3.2.19). Due to major clustering of taxa around the control carcass obscuring species names, a separate list of these species can be seen in Table 3.2.3. Clustering of species on the carcasses of the undisturbed graves, however, was dispersed due to decreased abundance and a low species diversity. Clear separation of the species involved in burial cases can be seen between the graves and the control carcasses with only a single overlapping species, *Sarcophaga* Meigen sp., occurring. Unique species below-ground included the staphylinid *Aleochara* sp. and the astigmatid mite *S. mycophagus*.

Fisher's exact test for association yielded differing results between the various undisturbed graves and control carcass. Since graves one, two, three and five did not yield any species during the trial, association with the control carcass was zero. From graves four and six, however, a P-value of 0.1700 and 0.0237 respectively was obtained. This indicates a significant association ($P<0.05$) between the control and grave six carcass, due to the presence of the *Sarcophaga* species in the grave, and a non-significant association ($P>0.05$) between the control and grave four carcass.

Pearson's correlation coefficient measured the strength of association between the various undisturbed grave carcasses and the control carcass, with regard to the presence/absence of species. Correlation coefficients were negative, being -0.4524 and -0.6726 for graves four

and six respectively. Since no species were sampled from the remaining graves the correlation of these graves with the control carcass could not be determined. The fact that the correlations between the control carcass and the grave carcasses were negative (when they could be calculated) suggests that a species is more likely to occur on a grave carcass if it is absent on the control carcass.

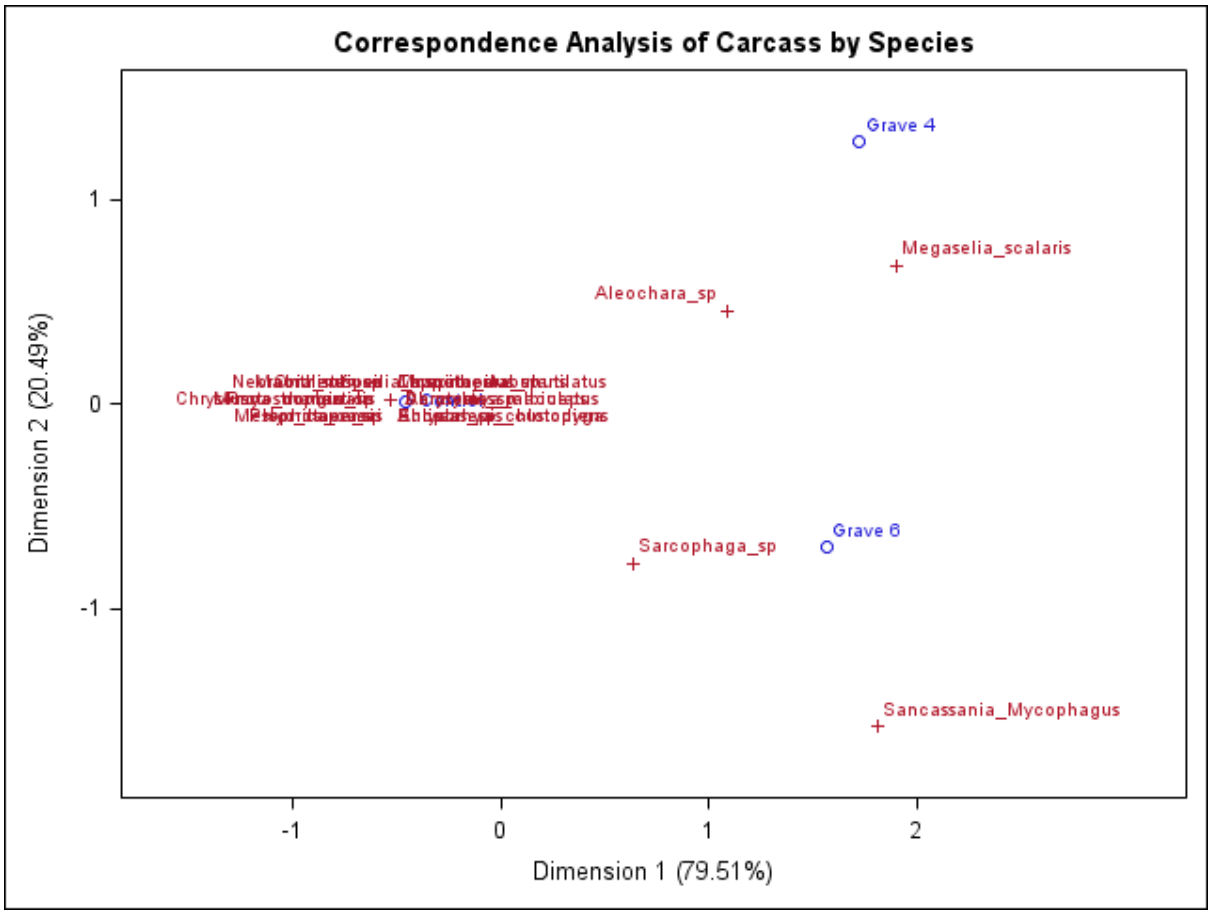

Figure 3.2.19: Correspondence analysis ordination showing clustering of species at the carcasses during the 2014/2015 summer trial

Table 3.2.3: List of species clustered around the 2014/2015 summer control carcass on correspondence analysis ordination.

Order	Family	Species
Diptera	Calliphoridae	*Lucilia* spp.
		Chrysomya marginalis
		Chrysomya albiceps
		Chrysomya chloropyga
	Muscidae	*Hydrotaea* sp.
		Muscina stabulans
		Musca domestica
	Piophilidae	*Piophila casei*
Coleoptera	Dermestidae	*Dermestes maculatus*
	Cleridae	*Necrobia rufipes*
	Silphidae	*Thanatopilus mutilatus*
	Staphylinidae	*Eulissas* sp.
	Histeridae	*Macrolister* sp.
	Scarabaeidae	*Ontherus* sp.
	Curculionidae	*Protostrophus* sp.
Hymenoptera	Formicidae	*Anoplolepis custodiens*
		Linepithema sp.
		Messor capensis
Orthoptera	Acrididae	*Acrotylus* sp.

3.2.7 General Overview

The rate of decomposition during the summer trial was seen to occur faster above-ground compared to below-ground (Figure 3.2.8). The control carcass was in direct contact with hot climatic conditions causing an increase in bacterial and enzymatic activity, as well as a high rate of arthropod succession. Kelly (2006 *unpublished*), Kolver (2009 *unpublished*) and Hoffman (2014 *unpublished*) observed similar results, with above-ground carcasses reaching advanced decay within 14 to 21 days. Buried carcasses of the present study, however,

underwent a decreased rate of decomposition. Heavy rainfall experienced over the trial period caused waterlogging of the graves which in turn decreased enzymatic and bacterial activity. No fungal growth was seen on any of the buried carcasses. Compaction of the grave soils after rainfall also ensured decomposition continued as the moisture present within the grave caused adipocere formation and putrefaction.

Due to decomposition occurring at a fast rate above-ground, the control carcass showed a faster rate of biomass loss as time progressed compared to the buried carcasses (Figure 3.2.9). Approximately 80% of the control carcass' biomass was lost at the end of the trial period. With the buried carcasses remaining in an isolated environment, and subsequent waterlogging of the graves occurring after heavy rainfall, less biomass was lost from the below-ground carcasses. During excavations, neither of the grave carcasses were found to have lost more than 50% of their biomass at the time of excavation. Janaway (1996) reported on the decreased activity of microbial and soil organisms in extremely waterlogged soils. High moisture levels within the graves during the trial period prevented increased microbial and soil organism activity, leading to a slower decomposition rate and less biomass loss from the carcasses compared to above-ground. Despite the high moisture content of the graves, anaerobic bacterial activity was still on-going allowing decomposition to continue.

Arthropod succession during the summer trial showed a high concentration of arthropods on the control carcass, with dipteran blowfly species being the most dominant. A reduced species diversity was noted on buried carcasses compared to above-ground as only two dipteran species were observed to colonise the carcasses along with a single staphylinid species and a mite species. Specific regions of the buried carcasses were colonised by these species showing aggregation as succession continued. While colonisation by these species occurred at a slower rate compared to above-ground, three different waves of succession were seen over the 60 day trial period. These different successional waves indicate the impact of grave soil, as well as heavy rainfall, on arthropod succession below-ground giving rise to differing successional time frames to determine PBI. Of interesting note during the trial was the sudden increase in abundance of adult blowfly species on the control carcass during the third week of the trial. By this time the control carcass had entered advanced decay and very few dipteran larvae were present on the carcass. The adult blowflies recorded on the control carcass were seen to lack colouration and fully developed wings (Figure 3.2.11), indicating that they had just emerged from pupae that were still present on or near the carcass. This

showed that little to no migration of third instar dipteran larvae had occurred due to heavy rainfall, and the remains of the carcass provided some form of shelter to the pupae.

The results obtained from the summer trial show differences occurring between above-and below-ground arthropod succession and decomposition. The isolated environment and waterlogging of the graves prevented faster rates of succession and decomposition from occurring compared to above-ground due to soil type of the graves.

SECTION 3.3:
Comparative analysis between winter and summer, with particular emphasis on below-ground decomposition and arthropod succession

With soil providing such an effective barrier in burial cases, factors affecting decomposition and arthropod succession are reduced and often substituted. Rodriguez & Bass (1985) stated that at depths greater than one foot (30 cm) degradation by insects and animals is inhibited greatly. At this depth decomposition odours are also unable to reach the surface easily (Vass *et al*. 2008), and soil impedes access to the cadaver. Cases of odour detection by certain insect species has been reported, however, with the blowfly species *Calliphora vicina* Robineau-Desvoidy detecting decaying mouse volatiles from shallow graves (Paczkowski *et al*. 2012). This has led to incidences of colonisation by certain arthropod species on buried carcasses and, subsequently, promotes decomposition of these remains. Comparisons revealed general differences in trends regarding temperature regulation, decomposition and arthropod succession during winter and summer. These trends provide insight into the effect of burial, within the central area of South Africa, applicable to forensic investigations.

3.3.1 Weather Conditions

Ambient temperatures recorded during the two seasonal trials were contrasting, with winter temperatures being lower and less rainfall occurring over the trial period (Figures 3.1.2 and 3.2.2). While winter ambient temperatures (13^0C to 28^0C) were lower in comparison to summer (21^0C to 44^0C), the temperature range of winter indicates a generally warmer season than winter temperatures experienced by Kelly (2006 *unpublished*), Kolver (2009 *unpublished*) and Hoffman (2014 *unpublished*). The vast difference in rainfall between the two seasons is also of significant note, with large amounts of rainfall causing fluctuations in ambient temperatures during the summer trial. While rain predominantly occurs during the summer months in central South Africa, a lower annual amount was experienced during the 2014 year compared to the expected average annual precipitation reported by MacKellar *et al*. (2014). Changes in these weather conditions bring about changes in arthropod behaviour and breeding patterns (Gennard 2007). This in turn affects the relevance of past seasonal studies in forensic investigations, leading to the necessity of ensuring changing climatic conditions are taken into account when estimations are formed (Ziervogel *et al*. 2014).

Stabilisation of temperatures, brought about by the insulating properties of soil, is a common factor affecting decomposition (Rodriguez 1993). Greater stabilisation occurs at depths greater than two feet (60 cm). Such stabilisation was seen in the disturbed grave one of both seasonal trials, with internal grave temperatures remaining in a small range of 10^0C to 20^0C (Figure 3.1.3) and 20^0C to 30^0C (Figure 3.2.4) for winter and summer respectively. Average internal grave temperature ranges of the undisturbed graves were found to be 13.18^0C to 18.32^0C and 26.36^0C to 32.28^0C for winter and summer respectively. No large fluctuations were recorded below-ground compared to above-ground. Temperature changes brought about by colonisation of arthropods has been recorded on above-ground cadavers, whereby intra-abdominal temperatures were increased by more than 5^0C above ambient temperatures (Simmons *et al*. 2010). The insulating property of soil becomes an advantage to the retention of heat generated by the buried carcass. This in turn affects decomposition of carcasses and the development rates of arthropods (MacMaster 2006).

Buried remains are subjected to a different array of environmental factors compared to remains above-ground. Many of the weather conditions experienced by carcasses on soil surfaces are impeded, giving rise to different abiotic and biotic components that impact decomposition and arthropod succession. However, one environmental factor was seen to circumvent the insulating behaviour of the grave soils and directly influence the buried carcasses. Rainfall, when in sufficient quantities, is able to breach the soil layer of graves and penetrate towards the lowest depths. This was seen during the summer trial whereby all the graves became waterlogged and the internal environment of each grave was subjected to a high moisture content. This in turn affected the internal summer grave temperatures (Figures 3.2.4 and 3.2.6), and shows the extent to which moisture can impact forensic investigations. Therefore, it must not be assumed that all environmental factors experienced above-ground will not have an effect on buried carcasses, but that the extent of their influence is dependent upon the depth of burial as well as their intensity.

3.3.2 Carcass Decomposition and Biomass Loss

Moisture content of the soil becomes a major factor affecting decomposition below-ground. Moisture levels, method of burial and soil type plays a large role in the formation of adipocere on carcasses and presence and abundance of soil organisms (Rodriguez 1997; Forbes *et al*. 2005b). While heavy rainfall and clay based soils ensured increased adipocere

formation during the summer trial, winter carcasses also showed high incidences of adipocere, but to a smaller extent, despite occurring in a drier soil environment. With less rainfall occurring during winter, the moisture present in the graves was due to decomposition fluids of carcasses being retained by soil particles. This in turn caused adipocere formation on the buried carcasses despite the drier conditions.

Soil type, influenced by moisture levels, can also affect rate of decay with previous studies obtaining varying results based on the geological locality of the graves (Turner & Wiltshire 1999; Wilson *et al*. 2007; Haslam & Tibbett 2009; Schotsmans *et al.* 2012). Hunter & Martin (1996) stated that compaction of soil, along with rainfall, will reduce availability of oxygen below-ground, reducing activity of chemical processes and decomposition. This in turn will cause an anaerobic environment to persist and restrict certain bacteria and fungi from surviving below-ground (Dent *et al*. 2004).

Vegetation has also been noted to impact decomposition below-ground, particularly in areas with deep penetrating root systems where the roots attempt to reach the nutrient rich carcass (Rodriguez 1997). While vegetation was present, the removal of grasses on the graves themselves, along with the fact that only grass species with short root systems occurred at the two localities, reduced the likelihood that decomposition was affected by vegetation. Removal of vegetation on the graves, along with freshly dug soil, became the main causative factor for graves becoming waterlogged during the summer trial.

Temperature affected decomposition as faster rates occurred below-ground in summer (Figure 3.2.8) compared to winter (Figure 3.1.8). Similar results were obtained during corresponding seasons by Meyer *et al*. (2013). As such, faster rates of biomass loss from the carcasses occurred during summer (Figure 3.2.9), compared to winter (Figure 3.1.9). Despite repeated burial, neither of the disturbed grave one carcasses from both seasons displayed an increase in the rate of biomass loss during the trial period. This deviates from results obtained by Carter & Tibbett (2008), where an increase in the rate of biomass loss of the carcass with repeated burial was shown.

While pH is an underlying condition of soil, its impact on decomposition varies according to level and duration of burial. Janaway (1996) found that in short-term burials pH does not have any significant impact upon decomposition, while over extended periods of time it can. Soil pH can vary due to a number of factors including soil type, moisture and decaying materials. Neutral or alkaline soils have been recorded to better preserve carrion (Bethell &

Carver 1987), while highly acidic soils can essentially dissolve carrion (Merbs 1997). Furthermore, soil acidity increases during the initial stages of decomposition due to decomposition fluids released by the carcass, as well as anaerobic bacteria causing fermentation (Gill-King 1997). Enhanced fungal growth has been observed in more acidic soil and can interact with buried remains, both directly and indirectly, causing an increased rate in decomposition (Rousk *et al*. 2009). This possibly explains the occurrence of a higher rate of fungal growth during the present winter trial as the soil of the graves was more acidic, in comparison to summer graves; however, it is uncertain whether fungal growth was a main driving force of decomposition on buried carcasses during the present study. High rainfall during summer prevented high acidity in the grave soils, and possibly provided a more neutral to alkaline pH which had an impact on fungal growth. The dynamics of fungi and fungal blooms become a possible forensic indicator in terms of PBI determination with fungal growth rates, linked to pH level, providing possible estimations as to duration of burial. However, more extensive research is required before the implementation of such indicators in forensic investigations. Soil pH levels have also been found to have an impact on the colonisation of bacterial species, and therefore decomposition, with higher pH levels leading to greater bacterial growth and activity (Gill-King 1997). Such an occurrence was seen during the summer trial, with possibly higher pH levels within the graves leading to faster rates of decomposition compared to winter.

3.3.3 Arthropod Succession

1. *Below-ground*

Exposed carrion resources are mainly exploited by members of Calliphoridae, leading to high levels of competition occurring on carrion between necrophagous species. Other dipteran species associated with carrion are often outcompeted and must find alternative food sources. This often leads these outcompeted species to colonise carrion resources that are inaccessible, or otherwise unfavourable, to calliphorid species (Lundt 1964; Payne *et al*. 1968). Despite the barrier effect of soil, arthropods were still able to colonise buried carcasses during winter and summer. In most situations, factors pertaining to burial depth, soil type, wrapping of a carcass and ease of soil penetration affect colonisation rate of buried carcasses. Unlike the results obtained by Pastula (2012 *unpublished*) during a summer season at a depth of 60 cm, colonisation did not occur within seven days after burial, during either of the present trials,

but instead occurred between 14 and 21 days. Differing geological and environmental conditions are the main causative factor for such results, as soils of the graves in the present study consisted of more sand and clay compared to a more sand and silt composition in the study by Pastula (2012 *unpublished*).

Results obtained from the present study found Phoridae being the most dominant species during winter. With certain Phoridae species being able to colonise buried carrion up to 1.8m below-ground (Merritt *et al*. 2007), it is of no surprise that species of this family were recorded during these trials. *Megaselia scalaris* and *Conicera tibialis* adults and larvae were sampled during the winter trial from day 21 onwards, and only *M. scalaris* adults were sampled during the summer trial from the same day. It is uncertain as to why *C. tibialis* did not occur during the present summer trial, as it has been sampled during summer months in previous studies (Pastula 2012 *unpublished*). It is thought that waterlogging of the summer graves was the main factor preventing colonisation by this phorid species. The sampled phorid species had successfully colonised all undisturbed grave carcasses from day 21 of the trials, with the exception of the disturbed grave carcasses and the undisturbed summer grave five carcass. The main reason for no colonisation occurring on the disturbed grave one carcasses was the high level of disturbance caused by periodic excavations, indicating that Phoridae species are likely susceptible to disturbance on buried carcasses. The lack of colonisation on the undisturbed summer grave five carcass was due to the specific soil composition of the grave, coupled with high rainfall, which caused the grave to become sealed and effectively inaccessible to arthropods. Motter (1898) found phorid larvae remaining on buried carrion for two months after death, correlating with the results obtained during the winter trial. Adult *C. tibialis* are reported to be only associated with remains that have been buried for at least a period of months and, in certain cases, years (Byrd & Castner 2010). However, the occurrence of this species, both adults and immatures, 21 days after placement suggests a reduced time frame in which *C. tibialis* is able to colonise buried remains.

Colonisation by *Muscina stabulans* larvae on day 60 in the undisturbed winter grave six provided evidence as to the ability of this Diptera species to colonise cadavers at 60 cm at a much later stage, bringing about a second cycle of succession. The absence of *M. stabulans* specimens during the summer trial indicates a possible inability of this species to colonise waterlogged graves. A similar secondary succession cycle was seen in the undisturbed summer grave six, with two successional waves occurring, after initial colonisation, by a

sarcophagid species and the mite species *Sancassania mycophagus*, leading to a total of three succession waves occurring during the summer trial.

While dipteran immatures are often found in cavities and mucous membranes of a carcass above-ground, the larvae of dipteran species sampled during both seasons from buried carcasses found them on the legs, hooves and stomach underneath the carcass, similar to results obtained by Pastula (2012 *unpublished*).

A species from the genus *Sarcophaga* was also seen to colonise buried carcasses during summer, with empty pupal casings being sampled from the soil above the carcass on day 60 in grave six. This indicated that a full life cycle had taken place below ground. A similar occurrence was seen during the winter trial, with phorid pupae being sampled from the undisturbed grave six carcass, but no emergence of adults had yet occurred. The presence of a sarcophagid species on the buried summer carcass differs from results obtained by Pastula (2012 *unpublished*), where no flesh fly species occurred on buried carrion at 60 cm. It is hypothesised that, in order to reach the buried carcasses, sarcophagid species larviposited on the soil surface, at which point the larvae burrowed down to the carcass to feed. Such an occurrence was seen by Rodriguez & Bass (1985) who witnessed flesh fly species larvipositing near soil cracks after heavy rainfall and the immatures burrowed down to the carcass. Moist soil of the graves possibly assisted larvae to burrow as a single, first instar sarcophgid larva was found in the soil of the disturbed grave one during summer, one day after heavy rain, and not on the carcass itself, indicating this species ability to burrow to buried remains. A lack of moisture in the winter graves, therefore, indicates as to why this species was not present on buried carcasses during this season, as larvae could not burrow effectively through such a dry soil environment. High moisture levels may impede colonisation of other dipteran species, such as *M. stabulans*, due to high incidences of larvae drowning, particularly in clay soils. This explains the absence of *M. stabulans* during the summer trial.

Adults of *Leptocera* sp. were found to occur only during the winter trial on the undisturbed grave six carcass (day 60), and no immatures were found. Members of *Leptocera* have been found to be associated with fungal growth, and certain species of this genus are involved with the nutrient cycle of the environment (Buck 1997). Fungal growth has been found to be associated with the pH levels of soil, with more acidic soil enhancing fungal growth compared to alkaline soil (Rousk *et al*. 2009). The presence of fungal growth on buried

carcasses during the winter trial may explain the occurrence of this *Leptocera* sp., as well as its absence during the summer trial where no fungal growth was seen on the buried carcasses. The lack of immatures on the winter grave six carcass also brings the question as to this species role in burial cases. The lack of *Leptocera* sp. immatures on the buried carcasses may be explained by this species being outcompeted by the adults and larvae of the Phoridae species and *M. stabulans* larvae, however its exact feeding behaviour is unknown. Another explanation may be linked to the possible association of this *Leptocera* sp. with fungal growth by using it as a food source and not using the carcass as a resource for reproduction. Thus, there would be no need for immatures to be present. The possible role of this *Leptocera* sp. with fungal growth on buried carcasses may prove useful during burial cases in winter during certain time periods and in the presence of fungal growth. However, its use may prove to be limited compared to those species that are able to reproduce and feed on buried carrion.

The astigmatid mite *S. mycophagus* was shown to occur in large numbers on only the disturbed grave one and undisturbed grave six carcasses during the summer trial and not in winter. Tsiafouli *et al*. (2005) found that soil microarthropod abundance and soil moisture shared a positive correlation, explaining the lack of mite species in the drier winter graves of the present study. *Sancassania mycophagus* is often found to feed upon the roots of asparagus plants, is a well-known pest of stored products and is classified as a scavenger species (Estebanes-Gonzalez & Rodriguez-Navarro 1991; Ostovan & Kamali 1995; O'Connor 2009). This brings into question the role of this mite species on buried carcasses. Certain mite species have been indicated as being potential indicators of PMI with changes in mite populations over time providing possible correlations in time since death (Goff 1989).

While the role of dipteran species has been extensively documented in forensic cases, certain situations occur whereby access to cadavers by insects is severely limited (Perotti & Braig 2010). In such situations mite species have been seen to occur in greater abundance and in various waves of succession. Although certain mite species already occur on healthy living humans, other species arrive after death and are able to colonise a cadaver at the same rate as insects and other arthropods (Perotti & Braig 2010). These species have been observed to arrive on a cadaver via airborne currents, as phoretics on insects and arthropods coming into contact with the cadaver, and walking (Perotti & Braig 2009; Perotti *et al*. 2010). Various types of mite species have been sampled from numerous cadavers under differing conditions. Such types include galcyphagid mites from a female carcass buried for 28 years in an unsealed casket (Merritt *et al*. 2007), indoor mites sampled from cadavers indicating place of

death (Frost *et al*. 2010) and astigmatid mites sampled en mass on an insect-free corpse (Russell *et al*. 2004). Results obtained from the present summer grave carcasses differed from the results obtained by Russell *et al*. (2004). While the astigmatid mite species *S. mycophagus* did occur in high abundance, the carcass itself was not insect-free due to the occurrence of adults and larvae of the staphylinid *Aleochara* species seen predating on the mites. It is uncertain, however, if these species colonised the carcass simultaneously or if the presence of *S. mycophagus* attracted the *Aleochara* species. As such, more research is required in order to properly establish the interaction of this mite species in burial cases.

While no coleopteran species were sampled below-ground in winter, due to colder conditions, a species of staphylinid belonging to the genus *Aleochara* was sampled in summer in large numbers. Despite being abundant on the carcass, adults of this species were seen to prey on mites and adult phorids and not on the carcass itself. Such an occurrence was seen by Lundt (1964), Van Laerhoven & Anderson (1999) and Corrĉa & Mourae (2014), in which similar beetles were commonly predacious on dipteran eggs and larvae below-ground. Larvae of this genus are generally considered parasitoids of dipteran pupae (Klimaszewski & Jansen 1993), with one species, *Aleochara bilineata* Gyllenhal, being used as a control agent of the cabbage maggot, *Hylemya brassicae* (Bouché) (Read 1962). Furthermore, some larvae of *Aleochara* species have been identified as being scavengers on decomposing remains (Anton *et al*. 2011). It could not be established during this study as to the exact feeding behaviour of the *Aleochara* sp. larvae sampled, and further studies are required in order to properly ascertain this. The presence of predacious Coleoptera below-ground also raises the issue of factors affecting arthropod succession and in turn PBI estimation.

2. *Above-ground*

High abundance of coleopteran species on above-ground carcasses during the two seasons consisted mainly of *Dermestes maculatus* adults and larvae and *Necrobia rufipes* adults. Both species are known to be associated with drier stages of decomposition (Byrd & Castner 2010), and also predate upon immature stages of Diptera. Due to a drier climate, low humidity and low temperatures, the winter control became increasingly dry and in a state akin to mummification within the first few weeks of the trial. The extent of this dryness provided a beneficial food source for *D. maculatus* individuals, allowing it to feed and breed on the carcass from day 14 onwards during winter. In contrast, colonisation by coleopteran species

coincided with the later stages of decomposition and only became more extensive after 21 days during summer. Similar occurrences have been documented before by Hinton (1963), Cloud & Collison (1986) and Smith (1986). While *D. maculatus* is not considered a winter species (Wang *et al*. 2008), above average ambient temperatures allowed for its occurrence during the winter trial. Breeding studies conducted by Richardson & Goff (2001) concluded that the lowest viable temperature *D. maculatus* is able to breed at is 20^0C. This correlates with ambient temperature data during the winter trial, with the majority of days having an ambient temperature of higher than 20^0C (Figure 3.1.1).

Hide beetles are known to accelerate aggregation on a food source due to pheromones present in the faeces of the adults. This, in turn, facilitates the congregation of both adults and larvae (Jaskulska *et al*. 1987; Rakowski 1988). Dermestidae species possess 48 possible larval instars, 16 times more than that of dipteran species (Boucher 1997 *unpublished*). Numerous larvae occurred on the winter control carcass at differing stages of development, along with the occurrence of previous larval casings. This indicated that moulting had taken place. This brings to question the potential of utilising such a species in forensic investigations, particularly during winter months when dipteran activity is low and the adults fail to oviposit and colonise successfully. Such a question becomes even more relevant due to climate change causing a shift in environmental conditions and the arrival time of this species. The regional studies conducted in the past by Kelly (2006 *unpublished*), Kolver (2009 *unpublished*) and Hoffman (2014 *unpublished*) show such a change in arrival time, with individuals of this species arriving during the later stages of decomposition. Ambient winter temperatures recorded during these past studies were generally lower (15°C on average) than those recorded during the present winter trial (average of 22°C). The arrival time of *D. maculatus* in past studies show a later colonisation occurring after 21 days, while colonisation occurred from 14 days onwards in the current study. However, more research is needed regarding the life-cycles and general biology of beetles if it is to be applied to the same extent as Diptera in the field of forensic entomology.

3.3.4 Statistical Analysis

Statistical analyses provided information on the significance of association between the control and grave carcasses. Correspondence analysis ordination showed major clustering occurring around both winter (Figure 3.1.19) and summer (Figure 3.2.19) control carcasses

due to the high species diversity and abundance, while those species involved in below-ground cases were shown to be separate in isolated clusters. Few instances of species overlap, where the respective species were present on both above- and below-ground carcasses, were shown to occur for both winter and summer (Figures 3.1.19 and 3.2.19). The Phoridae species *M. scalaris* and *C. tibialis*, the Staphylinid *Aleochara* sp. and the astigmatid mite *S. mycophagus* were shown to be of particular importance on buried carcasses, with the Phoridae being the most dominant during winter (Figure 3.1.14) and the staphylinid and mite species being the most dominant in summer (Figure 3.2.13). The consistently observed negative correlations between the controls and graves of winter and summer suggest a higher probability of a species occurring on the buried carcasses if it had not occurred on the control carcass, than the probability of a species occurring on the buried cadavers if it had occurred on the control. These results could be used as a starting point for developing a full successional pattern in below-ground cases to encompass a variety of ecological factors and environments throughout South Africa.

CHAPTER 4

Forensic Implications and Predictive Analyses

SECTION 4.1:

Baseline characteristics of decomposition stages and the main factors influencing it

4.1.1 Characteristics of Decomposition Stages and the Factors Influencing Decomposition

Previous regional above-ground forensic entomology studies by Kelly (2006 *unpublished*), Kolver (2009 *unpublished*) and Hoffman (2014 *unpublished*) determined the various stages of decomposition encountered based on characteristics described by Payne (1965) and Anderson & Van Laerhoven (1996) (Table 4.1.1). Such characteristics rely on visual cues coupled with the presence and effect of arthropods on a carcass. While these characteristics were utilised as a baseline for the purpose of this study, various differences were noted between above- and below-ground decomposition stages.

Table 4.1.1: Characteristics commonly utilised to identify the stages of decomposition (taken from Payne (1965) and Anderson & Van Laerhoven (1996)).

Stage of decomposition	Description of characteristics commonly used
Fresh	Commences once carcass is placed at study site. Oviposition by primary blow fly species commonly occurs.
Bloated	Carcass inflates due to build-up of gases within body caused by anaerobic bacterial action. Gases able to escape from openings such as wounds and orifices. Body fluids leak from orifices as inflation occurs. Odour build-up around carcass.
Active Decay	Active feeding by arthropod species, primarily dipteran maggots, on body cause deflation as gases escape through openings formed by feeding. Tissue becomes putrefied and fed upon by arthropods. Strongest odours encountered during this stage. Secondary blow fly species present.
Advanced Decay	Majority of flesh removed due to dipteran maggot feeding, and only dry flesh, skin and bones remains on carcass. Sections of skeleton visible but most parts still obscured by remaining skin and flesh. Odour begins to fade. Beetle species prevalent on carcass.
Dry Remains	Hair, bones and a few patches of skin remain on carcass. Skeleton mostly visible and devoid of flesh. Very few arthropod species present.
Transition	Transition identified when carcass is between two stages of decomposition and signs of both stages are present.

Some of the characteristics of the decomposition stages experienced by the buried carcasses, during the present study, differed in regards to the extent of inflation and putrefaction. These characteristics were pronounced during the hot, summer months.

Arthropod activity is generally at a peak during the summer season, with large numbers of dipteran larvae colonising decaying carcasses, resulting in an increased rate of decomposition and the prevalence of the active decay stage. Intense heat also increases internal enzyme and bacterial activity within a carcass (Carter *et al*. 2008), resulting in a marked inflation of the body and a release of large amounts of gas during the bloated stage (Byrd & Castner 2010). In contrast, a decrease in arthropod activity is often experienced during winter, which limits the rate of active decay (Byrd & Castner 2010). Since active decay is often reliant upon active feeding by sarcosaprophagous arthropods (Benecke 2004), a slower rate of decomposition is experienced. While inflation of a carcass can occur during the winter season, the extent of such inflation is less due to lower temperatures causing decreased enzymatic and bacterial activity.

The effect of environmental conditions can also give rise to variations in decomposition characteristics, with preservation methods (confinement and concealment of carcasses), locality, altitude and circumstances of death affecting the rate of decay (Byrd & Castner 2010; Pope 2010 *unpublished*). Such variation prompted the investigation into the current study to determine the impact of concealment methods, namely burial, on decomposition. Instances of cadavers placed within buildings and sealed containers have led to slower rates of decomposition, due to inaccessibility of sarcosaprophagous arthropods and unique microenvironmental conditions (Anderson 2011). Byrd & Castner (2010) noted that burial conditions have been found to impede access to cadavers along with a reduction in abiotic factors that can affect decomposition and biomass loss. With varying depths affecting arthropods detecting and accessing buried carrion, difficulties in PMI estimations become more prevalent (Catts 1992). Such difficulties lead to a reliance on research scenarios, by which proper estimations can be made in regards to soil type, environmental conditions and depth. This, in turn, may assist investigators to make more accurate estimations based on these specific scenarios, as opposed to reliance upon a more general scenario.

SECTION 4.2

Predicted patterns of below-ground decomposition based on observations at 60 cm

4.2.1 Review of Winter and Summer Below-ground Decomposition

Differences between above- and below-ground decomposition was noted during the winter (Section 3.1) and summer (Section 3.2) trials. These differences pertained not only to rate of decomposition but the characteristics of the various stages. Major differences were noted during the stages of bloat and active decay, whereby a number of the characteristics observed above-ground were absent on the buried carcasses. The stages of decomposition recorded above-ground will not be discussed here, due to the majority of past studies involving decomposition above-ground, and are only used in contrast to below-ground decomposition. The main emphasis of this section is to highlight the process of decomposition on buried carcasses.

1. *Winter*

   Decreased rates of decomposition were noted between the above- and below-ground carcasses in winter (Figure 3.1.8, Section 3.1). Specific characteristics were noted in regards to the decomposition of buried carcasses in the winter trial (Table 4.2.1). While no differences were noted during the fresh stage of decomposition, one difference was noted during the bloated stage between the above- and below-ground carcasses. The buried carcasses did not undergo the characteristic of inflation, which is the main indication of bloat, caused by the release of gases by anaerobic bacterial action (Table 4.2.1). In order to properly ascertain the current stage of decomposition for buried carcasses additional characteristics were utilised. Such characteristics included presence of fluids leaking from the body, the audible escape of gas and discolouration of the body. During active decay above-ground, the body deflates due to arthropod feeding, among other things, causing escape avenues for built up gases. According to Byrd & Castner (2010), active decay is the most prevalent stage of decomposition during winter months. While deflation was evident on the above-ground carcass during the winter trial, it did not experience the rapid tempo of deflation observed on the above-ground summer carcass. A similar form of deflation was noted on the buried carcasses. However, the extent of such deflation was not as described by Payne (1965) and Anderson & Van Laerhoven (1996) due to the extent of initial inflation being extremely low. Another factor affecting this deflation was a decreased arthropod diversity and abundance on the buried carcasses. With the Phoridae

species being small in stature, large quantities of food are not required to ensure development, leading to a low rate of feeding occurring on the buried carcasses and thus a slow rate of deflation. Cold winds and mild ambient temperatures during winter caused the above-ground carcass to become dry during active decay, effectively causing the carcass to undergo a form of mummification. Putrefaction was more prevalent on buried carcasses during active decay than on the above-ground carcass. A large degree of skin slippage was also noted on the buried carcasses, together with fungal growth (Table 4.2.1).

2. *Summer*

During the summer trial, increased rates of decomposition were noted for both above- and below-ground carcasses (Figure 3.2.8, Section 3.2), compared to winter (Figure 3.1.8, Section 3.1). Specific characteristics were noted during summer in regards to the decomposition of buried carcasses (Table 4.2.1). Only four stages of decomposition occurred below-ground during the 60 day trial, while five stages occurred above-ground. While the fresh stage of decomposition lasted less than a day above-ground, due to hot weather conditions, the fresh stage lasted for a few days on the undisturbed buried carcasses. No major differences were noted in characteristics for the fresh stage between the above- and below-ground carcasses. The bloated stage followed on rapidly from the fresh stage above-ground. However, no such stage was recorded below-ground. This absence of the bloated stage below-ground was caused by heavy rainfall during the first week of the summer trial, which led to compaction of the clay soils in the graves. This occurrence alone provides an example of what factors are able to affect decomposition below-ground due to soil type and weather conditions. When the carcass of grave two (day 7) was excavated, a day after heavy rainfall, the body was noted to be flattened and extensive adipocere formation and putrefaction was seen (Table 4.2.1). Such extensive adipocere formation was not recorded on the above-ground carcass, despite it being fully exposed to rainfall. Adipocere formation was due to the clay based soils of the graves that became waterlogged, effectively increasing the hydrolysis rate of fatty tissue on the buried carcasses. As time progressed, the excess moisture drained away slowly and the carcasses took on a husky appearance. Due to soil compaction in a number of the graves, parts of the bodies easily broke off as the carcasses transitioned into advanced decay. Advanced decay was the most prevalent stage of decomposition on the buried carcasses during summer months (Figure 3.2.8, Section 3.2). Soil compaction on the buried carcasses was a common occurrence after rainfall due to the clay composition of most graves. While the

carcasses in the disturbed grave and the graves excavated on days 7, 14, 21 and 30 reached advanced decay at the end of the 60 day trial period, the carcass excavated on day 60 remained in active decay. All graves consisted of a clay-loam mixture in varying concentrations, but grave six (day 60) possessed the highest clay concentration. This soil composition effectively prevented adequate drainage of remaining water, denoted by the still present adipocere and putrefaction on the grave six carcass.

The main differences between winter and summer decomposition included rate of deflation of the carcasses along with abundance of fungal growth. Similarities included the presence of large amounts of putrefaction, skin slippage and discolouration of tissue. Another major difference was the absence of advanced decay and the bloated stage during the winter trial and summer trial respectively. From these observations it can be seen that certain differences do occur below-ground in terms of the characteristics used for identification of decomposition stages. While some of these differences are relatively small, the absence of certain characteristics brings the requirement of further studies to the fore. These characteristics, however, do have potential in being utilised for forensic cases under specific burial conditions.

Table 4.2.1: Decomposition characteristics of the undisturbed buried carcasses during the 2014 winter and 2014/2015 summer trial.

Stage of decomposition	Characteristics recorded (2014 winter trial)	Characteristics recorded (2014/2015 summer trial)
Fresh	• No odour present from carcass • Discolouration occurring on neck and stomach of carcass • No arthropod activity seen on carcass	• No odour present from carcass • Discolouration occurring on neck and stomach of carcass • No arthropod activity seen on carcass
Bloated	• No inflation of body occurred, compared to above-ground • Leakage of decomposition fluids from orifices seen beneath carcass • Small amounts of fungal growth seen of carcass • No putrefaction seen yet • No arthropod activity seen on carcass	*Stage of decomposition not experienced during summer trial*
Active Decay	• Large extent of putrefaction on carcass • Skin slippage prevalent on limbs and beneath carcass • Deflation of carcass minimal, compared to above-ground • Large amounts of fungal growth seen on carcass • Arthropod activity on carcass	• Large amounts of putrefaction on carcass • Skin slippage prevalent on limbs and beneath carcass • Deflation of carcass extensive due to soil compaction by heavy rainfall • No fungal growth seen on carcass • Arthropod activity on carcass
Advanced Decay	*Stage of decomposition not experienced during winter trial*	• Carcass extremely deflated • Carcass seen to take on husky appearance as water drained from grave • Parts of carcass seen to easily break off from main body • Arthropod activity on carcass

4.2.2 Review of Winter and Summer Below-ground Biomass Loss

Differences in biomass loss were noted during the winter (Section 3.1) and summer (Section 3.2) trials between above- and below-ground carcasses. Most differences pertained to the rate of biomass lost during the 60 day trial periods and the final biomass recorded for the carcasses. The biomass loss of above-ground carcasses will not be discussed here, due to the majority of past studies involving biomass loss above-ground, and is only used in contrast to below-ground biomass loss.

1. *Winter*

   A steady rate of biomass loss was recorded during the winter trial on all carcasses as the trial progressed (Figure 3.1.9, Section 3.1). Biomass was lost at a faster rate from the buried carcasses compared to above-ground, with the exception of the disturbed grave carcass. None of the buried carcass lost more than 20% of their biomass at the times of excavation (Figure 3.1.9, Section 3.1), but had still lost more biomass than the above-ground carcass at those specific times. An initial 5% increase in biomass within the first 3 days of the trial was noted on the disturbed grave carcass, caused by fluid absorption and retention by the carcass. The rate of biomass loss of this disturbed grave carcass then followed a similar pattern to the above-ground carcass, losing less than 30% of its biomass at the end of the trial period. Due to the high clay-loam composition of grave six, less than 5% of the biomass of the carcass excavated on day 60 was lost, corresponding with the decomposition stage recorded for this carcass.

2. *Summer*

   An exponential loss in biomass from the above-ground carcass was observed compared to the buried carcasses during the summer trial (Figure 3.2.9, Section 3.2). High ambient temperatures (Figure 3.2.1, Section 3.2) increased the rate of decomposition of the above-ground carcass and a higher abundance of arthropod species colonised the remains (Figure 3.2.10, Section 3.2), leading to more than 80% of its biomass being lost at the end of the trial period. The buried carcasses, however, lost biomass at a slower rate, with no carcass losing more than 50% of its biomass at the times of excavation (Figure 3.2.9, Section 3.2) due to waterlogging of the graves. The disturbed grave carcass lost biomass at the slowest rate due to the high level of disturbance experienced. Despite being undisturbed for the longest period of time, the carcass excavated on day 60 still possessed 70% of its biomass. The high clay composition of grave six, together with heavy rainfall causing waterlogging

of the grave, prevented a high rate of biomass loss, corresponding with the stage of decomposition recorded for this carcass.

A generally faster rate of biomass loss occurred on the below-ground carcasses during the winter trial compared to the above-ground carcass, as the soil layer of the graves provided a buffering effect against the cold climatic conditions. During the summer season, however, biomass loss occurred at a faster rate above-ground, due to the carcass being exposed to hot weather conditions and a greater arthropod abundance, compared to the below-ground carcasses. Biomass loss was also seen to occur at a faster rate below-ground during the summer trial compared to the winter trial. This is mainly due to heavy rainfall, causing compaction and waterlogging of the graves, and a higher temperature range in the summer graves compared to winter, causing an increase in anaerobic bacterial activity and putrefaction

4.2.3 Predicted Decomposition Patterns of Buried Carcasses at 60 cm

Using the rates of decomposition and biomass loss obtained during the winter and summer trials, predictive patterns can be estimated for undisturbed buried carcasses under the specific conditions, such as soil type and weather conditions, experienced during this study.

1. *Winter*

   During the winter trial, three types of soil mixtures were recorded at 60 cm depth. These comprised mostly of shale-sand (graves one, three and five) and clay-shale mixtures (graves two and four), with a single clay-loam mixture (grave six). As the winter trial progressed, variations in decomposition and biomass loss rates were observed between the clay-loam grave and the remaining graves. A slower rate of decomposition and biomass loss was experienced in the clay-loam soil mixture compared to the clay-shale and shale-sand mixtures during the trial. A greater amount of biomass was lost from the grave four (day 21) and five (day 30) carcasses at the time of excavation, indicating a faster rate of decomposition had occurred. The carcass of grave six lost less than 5% of its biomass by the time it was excavated compared to the biomass lost by the previously excavated carcasses. This is an indication of a decreased rate of decomposition occurring in a clay-loam soil. From this, predictive patterns of decomposition for buried carcasses within 60 days, during a dry winter season, were estimated based on soil type (Figure 4.2.1).

2. *Summer*

During the summer trial, the graves were comprised of only two types of soil mixture at 60 cm depth. Grave five contained a clay-loam-shale mixture while the remaining graves comprised mostly of clay-loam mixtures. Many of the graves consisting of clay-loam mixtures contained a high clay concentration. As the summer trial progressed, small variations were seen in regards to rates of decomposition and biomass loss between these two soil mixtures, as well as between different concentrations of the clay-loam mixture. The less concentrated clay-loam mixtures and the clay-loam-shale mixture followed similar rates of decomposition. The carcass in grave five (clay-loam-shale mixture) was seen to lose less than 40% of its biomass, compared to the previously excavated carcasses in graves three and four (clay-loam mixtures). This indicated a slightly slower rate of decomposition and biomass loss from the clay-loam-shale grave carcass. Grave six was comprised of a very high clay-loam concentration, both at 30 cm and 60 cm. The carcass of this grave lost less biomass than the previously excavated carcasses from the other graves, effectively losing no more than 30% of its biomass after 60 days. Furthermore, during its excavation, the carcass was noted to only be in active decay while the previous excavated carcasses were already in advanced decay. From this, predictive patterns of decomposition were estimated based on soil type and concentration, within a 60 day period, and during heavy rainfall (Figure 4.2.2).

Despite these estimations, further studies are required in order to properly predict the complete decomposition range that occurs below-ground during different seasons, and in differing soil types, in central South Africa. Deviations from these predictions can, and will, occur based on environmental conditions as well as soil type. From the results obtained it can be seen that even within the relatively small area the study was conducted in, soil type was variable and became one of the main driving forces of below-ground decomposition. Although this seemed to be the case during this particular study, further investigation is required to determine the variable impact of pH, moisture content, nutrient levels and soil particle size in particular on decomposition and arthropod succession. Soil type, linked together with weather conditions and depth of burial, not only affects decomposition but also the rate of arthropod succession and detection. It thus becomes imperative that regional studies be considered in order to clarify differences in decomposition below-ground throughout South Africa and under differing climates. Regardless, the information provided here can be utilised as a starting point for future studies in burial cases.

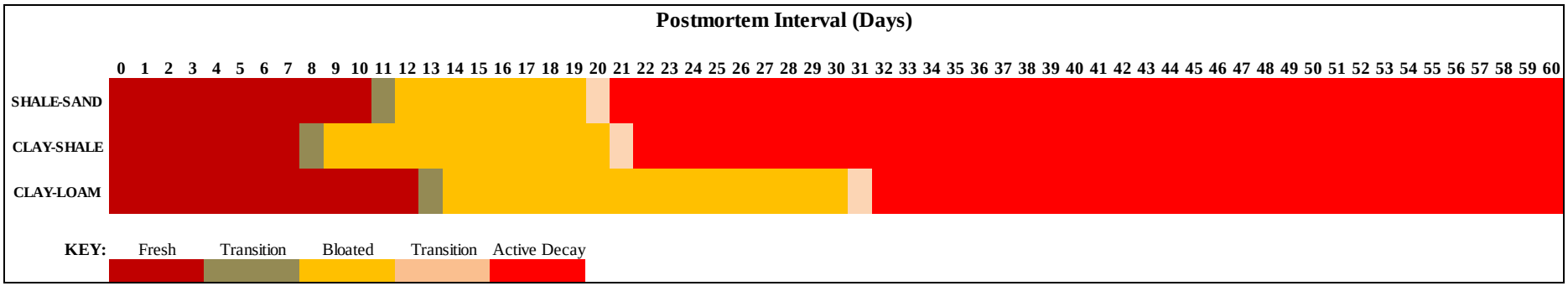

Figure 4.2.1: Estimated decomposition rates of undisturbed buried carcasses during a dry winter season in three different soil types, over a 60 day period.

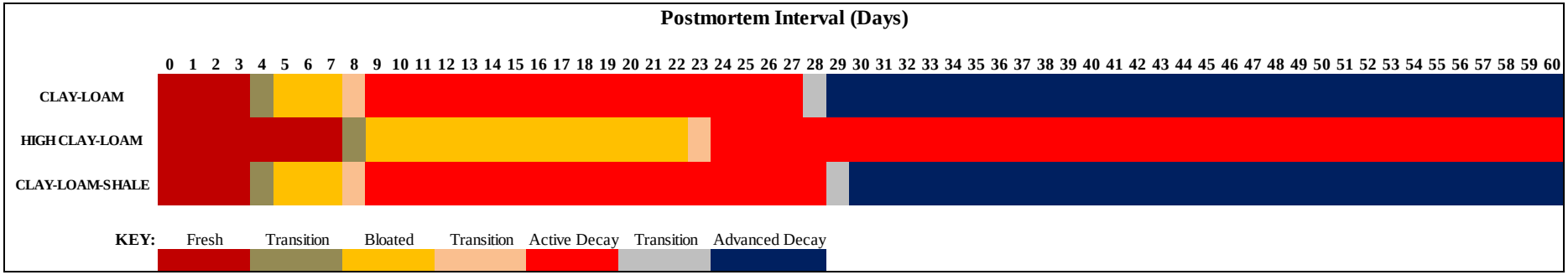

Figure 4.2.2: Estimated decomposition rates of undisturbed buried carcasses during a summer season with heavy rainfall in three different soil types, over a 60 day period.

SECTION 4.3

Predicted patterns of below-ground arthropod succession based on observations at 60 cm

4.3.1 Review of Winter and Summer Below-ground Arthropod Succession

Differences between above- and below-ground arthropod succession was noted during the winter and summer trials. Such differences pertained to abundance and diversity of arthropod species that were able to colonise or reach the buried carcasses. Colonisation of the buried carcasses was noted to occur by specific species. The arthropod succession recorded for above-ground will not be discussed here, due to the majority of past studies focusing on arthropod succession above-ground, and is only used in contrast to below-ground arthropod succession.

1. *Winter*

   Diptera was the only order to colonise all buried carcasses during the winter trial, while a single centipede specimen was also sampled from grave five (day 30) (Figure 3.1.14, Section 3.1). The rate of succession on buried carcasses was slower than above-ground, and those species occurring below-ground did not colonise the above-ground carcass. Colonisation was first seen during the excavation of grave four (day 21) when Phoridae *Megaselia scalaris* and *Conicera tibialis* adults and larvae were sampled from the carcass. It was estimated from these results that arrival of these species may have occurred between 14 and 21 days after burial since no colonisation had occurred on the grave three carcass (day 14). The larvae of these phorid species were seen to colonise specific areas of the carcass, particularly underneath the body where putrefaction was greater and, even then, only around the legs, stomach and head. Adults, on the contrary, were seen above and below the body in various areas. On day 30 of the trial, the same species were sampled from the grave five carcass (Figure 3.1.14, Section 3.1), occupying similar areas of the body as in grave four. Due to the higher abundance in grave five, compared to the previously excavated grave four, it can be estimated that colonisation took place at roughly the same time as on the previously excavated carcass. A single centipede individual was sampled from the grave five carcass, and was seen to prey on the phorid immatures. While centipedes have been observed preying upon blowflies and other fauna associated with carrion, the impact of a single individual on successional rates can be considered negligible (Gunn 2011). However, further research is required in order to

investigate the full impact of predatory individuals on succession rate in below-ground cases.

At the end of the trial period (day 60), abundance of the phorid species had again increased and two more dipteran species were sampled from the grave six carcass (Figure 3.1.14, Section 3.1). The two additional species sampled consisted of a *Leptocera* species (Family: Sphaeroceridae) and the muscid species *Muscina stabulans*. Only adults of the *Leptocera* sp. and larvae of *M. stabulans* were found, while adults, larvae and pupae of the phorid species were also sampled from the carcass. The late colonisation by these two species, *Leptocera* sp. and *M. stabulans*, signifies that different periods of succession can occur on buried carcasses. *Muscina stabulans* may be partially dependent on the rate of decomposition; however, the occurrence of the *Leptocera* sp. will coincide with fungal growth rather than decomposition stages.

2. *Summer*

A noted decrease in species diversity and abundance was seen on undisturbed buried carcasses during summer (Figure 3.2.13, Section 3.2), due to heavy rainfall, compared to the winter trial (Figure 3.1.14, Section 3.1). The rate of succession on buried carcasses was slower compared to above-ground, with specific species occurring. The first record of colonisation was on day 21 of the trial on the carcass of grave four. A small number of *M. scalaris* adults were seen moving around above and beneath the carcass, near the stomach and hind legs, but no immatures were found. A coleopteran species from the staphylinid genus *Aleochara* was also found on the grave four carcass, with adults and larvae being sampled. Adults of this species were seen to prey on the adult *M. scalaris* individuals, causing overlaps in aggregation sites on the carcass. It was estimated from the presence of arthropods on the grave four carcass (day 21), that colonisation occurred between days 14 and 21 as no arthropods were found on the grave three carcass on day 14. This estimation was supported by the data from the disturbed grave one carcass, when a first instar sarcophagid larva was sampled from the grave soil on day 18 (Figure 3.2.16, Section 3.2). Despite it not being the same species as that sampled from grave four, the presence of this larva still provides an indication for arthropod succession on undisturbed buried carcasses. No further colonisation occurred on the remaining undisturbed grave carcasses until the excavation of grave six on day 60 due to the effective sealing of grave five (day 30), caused by heavy rainfall and a high clay soil concentration, both on the surface and at the bottom of the grave. The highest diversity of arthropods was found on day 60 with a total

of four species occurring in grave six. Adult *M. scalaris* individuals were sampled from the grave but, once again, no immatures were found. Open sarcophagid pupae were found within the soil of the grave above the carcass, indicating emergence of adults and that a full life cycle had taken place within the grave. It is estimated that colonisation by sarcophagid larvae occurred between 30 and 40 days after burial due to further colonisation by this species being seen in the disturbed grave one between 33 and 40 days after burial (Figure 3.2.16, Section 3.2). A single mite species, *Sancassania mycophagus*, was found in large clusters on the stomach of the grave six carcass. The adults of the staphylinid *Aleochara* sp. were seen to prey extensively on these mite individuals. Larvae of this staphylinid *Aleochara* sp. were also seen roaming on the carcass, however, it is uncertain as to what they were feeding on.

From these results niche partitioning can be clearly seen occurring on the buried carcasses based on species present, their abundance and the current stage of decomposition. This niche occupation may prove useful in investigations of buried carcasses to simplify collection of entomological evidence for analysis.

4.3.2 Trophic Interactions Occurring Below-ground

Trophic interactions are often the driving force behind arthropod succession and can be linked to rate of colonisation. Trophic interactions, such as predation, can also lead to problems in PMI estimations during forensic investigations. It thus becomes necessary to determine what possible trophic interactions can occur during arthropod succession on a carcass so that they it be taken into account when using entomological evidence.

From the observations made during the study, trophic interactions were noted to occur between the various species colonising the buried carcasses. The buried carcasses represent a trophic and reproductive resource for the different arthropod species that colonise carrion. Ecological classifications have been proposed in forensic entomology to represent the fauna and their trophic roles on carrion. Leclercq (1975) proposed the classification of necrophages as those that feed from corpses, necrophiles as those that predate or parasitize on other arthropods, omnivores as those that feed from carcasses and prey upon other arthropods and incidentals as those that appear at random on the carcass. These classifications were utilised to identify the trophic interactions occurring below-ground on the buried carcasses.

During winter two trophic interactions were noted (Figure 4.3.1), namely necrophagous feeding by dipteran species and necrophilous predation by a centipede *Cormocephalus* individual. Phoridae species were seen to feed directly on the buried carcasses. Most species of the genus *Leptocera*, however, are commonly attracted to, and feed on, fungal growth on carcasses rather than on the carcass itself. Such fungi feeding organisms are known as mycophages. This indicates a possible secondary trophic interaction occurring. However, as the exact species of *Leptocera* encountered during this study is unknown, it is unclear what its actual feeding behaviour was. As such this species has been grouped separately under necrophages due to the possibility that they feed on a substance associated with the decay of the body, and colonised the buried carcass of grave six (Figure 4.3.1).

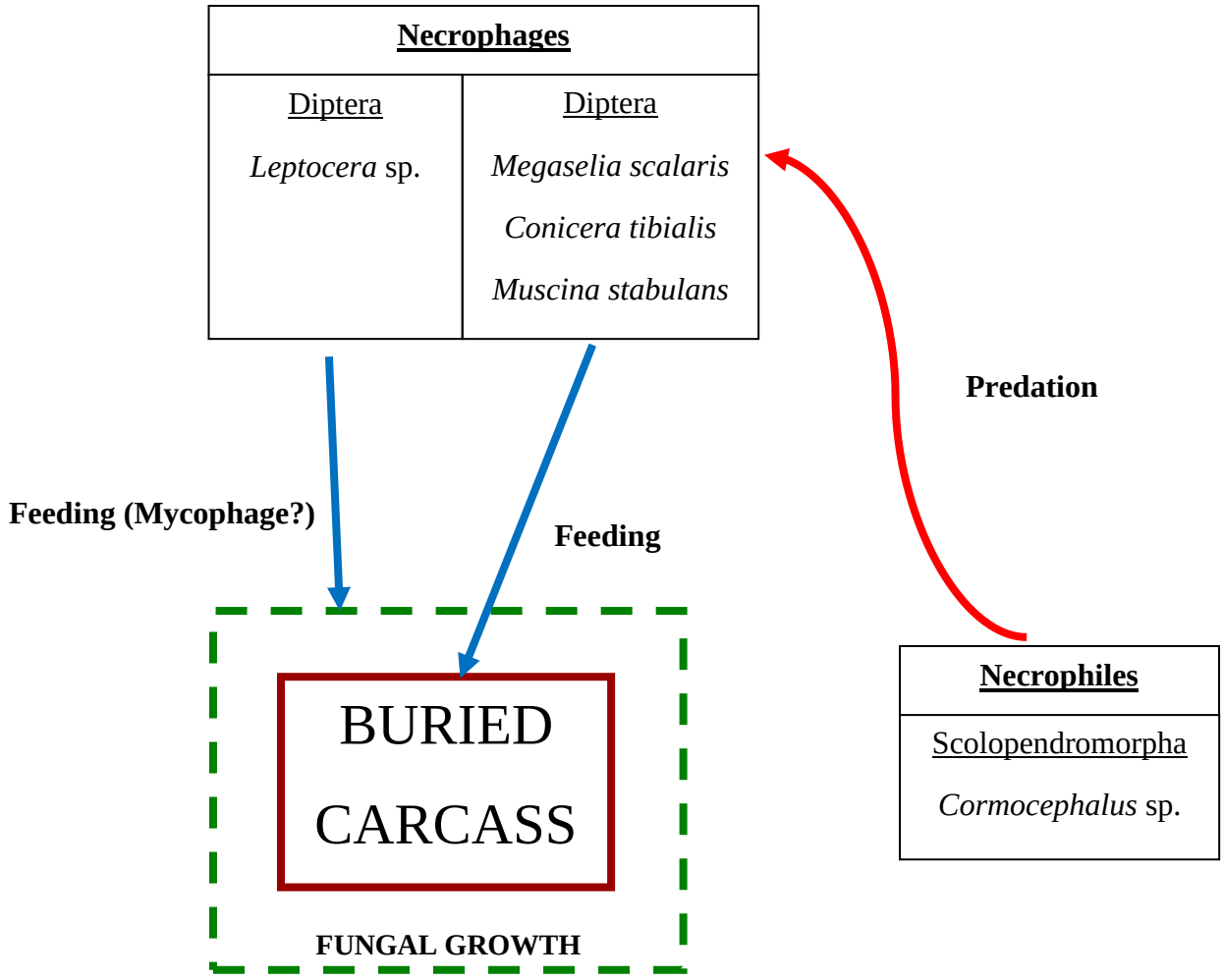

Figure 4.3.1: Trophic interactions recorded on buried carcasses during the 2014 winter trial.

Similar trophic interactions were noted to occur during the summer trial, with dipteran species, as well as a mite species, feeding on the buried carcasses and a coleopteran species

preying on these necrophagous species (Figure 4.3.2). The mite *S. mycophagus* was seen to feed extensively on the buried carcasses and aggregated in large clusters. Sarcophagid larvae later colonised the carcass of grave six (day 60) and began to feed extensively on the carcass. Only phorid adults were seen on the buried carcasses, compared to both adults and larvae present during winter. Feeding by these individuals was not as extensive due to a lower abundance. Staphylinid *Aleochara* sp. adults preyed upon adult phorids and mite individuals extensively during the trial. The feeding behaviour of the *Aleochara* sp. larvae was unknown, however, as they were only seen to roam on the buried carcasses. While most adult *Aleochara* species have been identified as predators, with larvae being parasitoids of dipteran pupae (Klimaszewski & Jansen 1993), some larvae species of this genus have been reported as being scavengers on decomposing remains (Anton *et al*. 2011). However, the exact identification of the *Aleochara* species found during this trial is unknown. Therefore, it is uncertain whether the larvae of this species are scavengers or parasitoids. Thus, due to uncertainty, two roles were assigned to *Aleochara* larvae, as scavengers (necrophages) on the remains and as predators/parasitoids (necrophiles) of Diptera associated with the remains (Figure 4.3.2). It may also be possible that the larvae can act in both roles depending on the species.

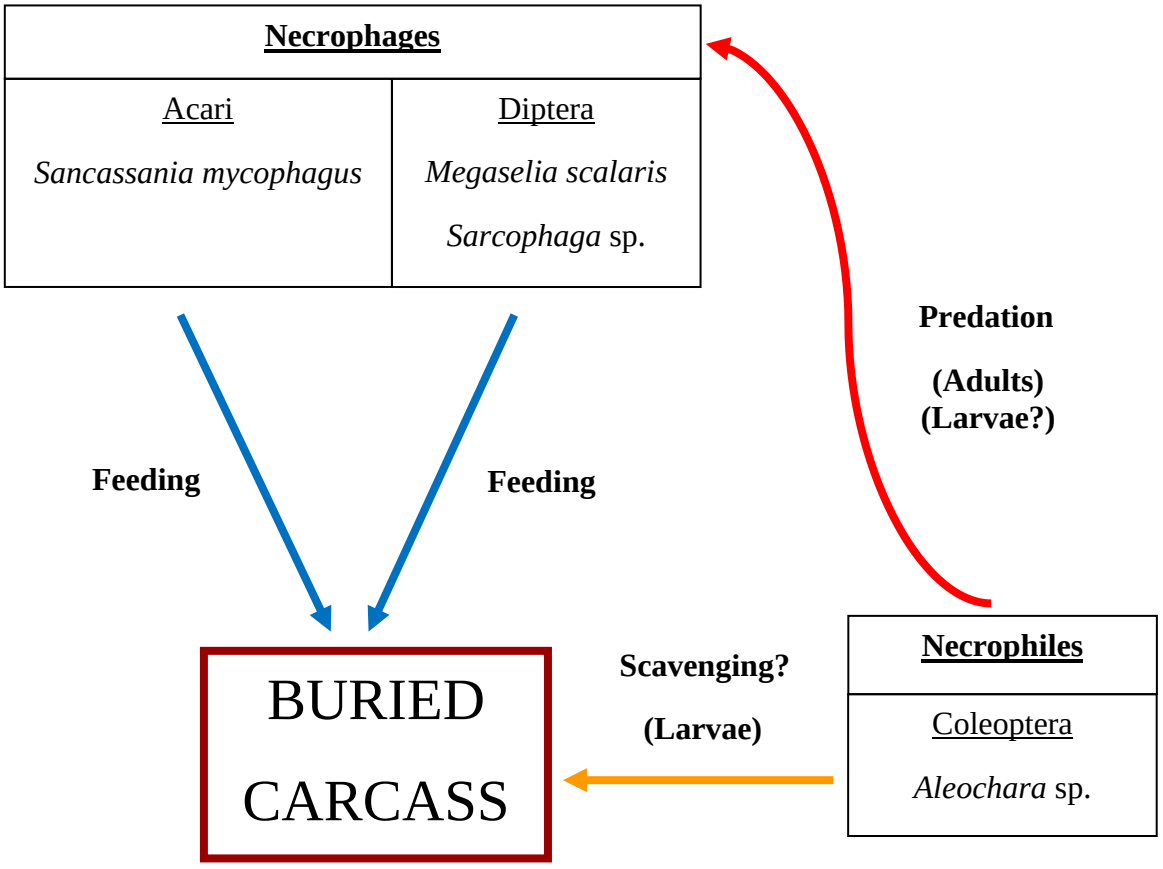

Figure 4.3.2: Trophic interactions recorded on buried carcasses during the 2014/2015 summer trial.

These trophic interactions indicate what biotic factors may affect PBI estimations, particularly in regards to predation. It may also prove useful as to the usage of indicator species in order to locate buried carrion via specific cues. Investigators must also take into account the subsequent interaction of these indicators species with buried carrion, colonising arthropods and the associated conditions of the body when making estimations.

4.3.3 Predicted Arthropod Succession Patterns on Buried Carcasses at 60 cm

Using the rates of arthropod succession recorded during the winter and summer trials, as well as trophic interactions, predictive patterns can be estimated for undisturbed buried carcasses under specific conditions, such as soil type and weather conditions, experienced during this study.

1. *Winter*

   During the winter trial, two succession waves were noted on the undisturbed buried carcasses (Figure 4.3.3). Initial colonisation occurred between 14 and 21 days after burial with the two Phoridae species *M. scalaris* and *C. tibialis* present on the body. Due to larvae of these species being present it can be assumed that colonisation occurred relatively early during the six day gap period between the grave three (day 14) and grave four (day 21) excavations (Figure 4.3.3). From the results obtained it was concluded that these Phoridae species remained on the colonised buried carcasses and reproduced, as a higher abundance of larvae of these species was recorded during the grave five excavation. Thus, abundance will continue to grow on colonised buried carcasses until the available food source is depleted. Re-colonisation by these phorid species may also be possible depending on the accessibility and state of the buried carcass.

   A second succession wave was recorded on the undisturbed buried carcasses by day 60, whereby adults of a *Leptocera* species and larvae of the muscid species *M. stabulans* were sampled. It is estimated that colonisation by these species occurred between 30 and 60 days after burial. Based on the abundance of the adult *Leptocera* sp. and the size of *M. stabulans* larvae it can be further estimated that colonisation most likely occurred by day 45 as many of the *M. stabulans* larvae were only beginning to reach third instar level and abundance of the *Leptocera* sp. was high (Figure 4.3.3). Soil type can play an immediate role in the delay of detecting a buried carcass. It can therefore be said that colonisation is

not fully dependent on the decomposition stage of the buried carcass, but more on the properties of the barriers (soil) that delay arthropods from colonising. Due to the study only lasting 60 days it is uncertain how long the *M. stabulans* larvae remained on the buried grave six carcass. It is possible that colonisation by this species would have continued for another two weeks before migration and pupation occurred due to only a few larval individuals being in third instar stage.

2. *Summer*

During the summer trial, three succession waves were noted on the undisturbed buried carcasses (Figure 4.3.4). Colonisation of the carcasses was estimated to have first occurred between 14 and 21 days after burial. The first species recorded on the buried carcasses was the phorid species *M. scalaris* and a staphylinid *Aleochara* species. Due to the abundance of both species being relatively low, due to heavy rainfall and subsequent waterlogging of the graves, it can be estimated that initial colonisation occurred within the 6 day gap period between the grave three (day 14) and grave four (day 21) excavations (Figure 4.3.4). Two separate succession waves occurred by day 60 of the trial with the mite species *S. mycophagus* occurring in high abundance on the grave six carcass and open sarcophagid pupae being sampled from the grave soil above the carcass. Due to open sarcophagid pupae being present in grave six it can be estimated that colonisation occurred 30 days after burial, and emergence from the pupae occurred a few days before the excavation of the grave, indicating a second succession wave (Figure 4.3.4). This estimation is supported as larvae of this species were seen between 33 and 40 days after burial on the disturbed grave one carcass. Similarly, it can be estimated that the mite *S. mycophagus* also colonised the undisturbed grave six carcass 36 days after burial, as increases in abundance of this species was seen on the disturbed grave one carcass from day 39 onwards, indicating a third succession wave (Figure 4.3.4).

Despite these estimations, further studies are required to establish a large data base in order to properly predict arthropod succession below-ground during differing seasons in central South Africa. Deviations from these predictions can, and will, occur based on environmental conditions as well as soil type and depth of burial. For example, as grave depth increases, further delays in colonisation are more likely to occur. Furthermore, the results obtained during this study cannot be considered an indication of all arthropods that are able to colonise buried carcasses, as changes can occur based on locality and season. While most sampled species were found on the undisturbed grave six carcass (day 60) during both trials, which

was still in active decay for both winter and summer, it would be extremely difficult to establish a predictive succession time line for the process of colonisation during common climatic conditions. In order to properly establish this type of predictive succession, it becomes imperative that regional studies be considered in order to clarify differences in arthropod succession below-ground throughout South Africa under differing climates, and attempt to construct a full species list of arthropods able to colonise buried carrion. For this reason, the predictive analyses presented in this section are currently only applicable to dry winter seasons and summer seasons with heavy rainfall in central South Africa.

Family	Species	Postmortem Interval (Days)
		1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60
Phoridae	*Megaselia scalaris*	
	Conicera tibialis	
Sphaeroceridae	*Leptocera* sp.	
Muscidae	*Muscina stabulans*	

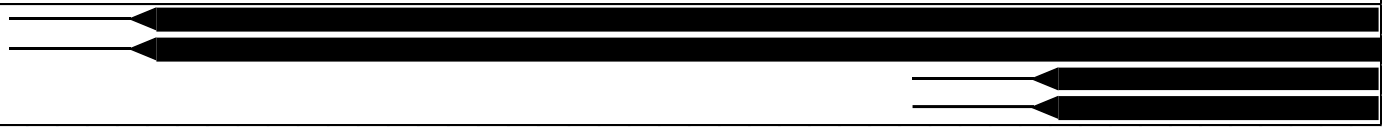

Figure 4.3.3: Estimated arthropod succession on undisturbed buried carcasses during a dry winter season in central South Africa, over a 60 day period.

Family	Species	Postmortem Interval (Days)
		1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60
Phoridae	*Megaselia scalaris*	
Sarcophagidae	*Sarcophaga* sp.	
Staphylinidae	*Aleochara* sp.	
Acaridae	*Sancassania mycophagus*	

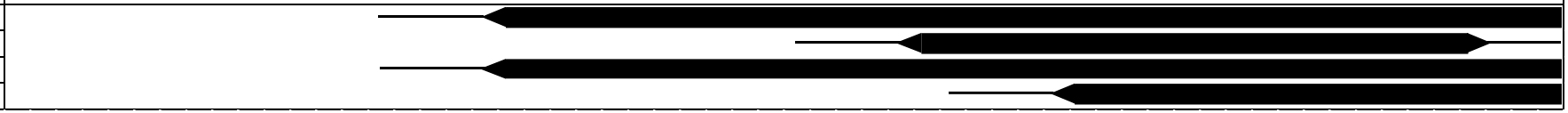

Figure 4.3.4: Estimated arthropod succession on undisturbed buried carcasses during a summer season with heavy rainfall in central South Africa, over a 60 day period.

SECTION 4.4

Practical implications regarding excavation methodology and collection of entomological evidence from a clandestine burial site

4.4.1 Review of the Methodology of Collecting Entomological Evidence at a Crime Scene

The following information, taken from Byrd et al. (2010), is discussed below.

Byrd *et al*. (2010) provides guidelines to the process of collecting entomological evidence at a crime scene which is briefly described here. For the full description of guidelines provided please refer to Byrd *et al*. (2010) "Collection of Entomological Evidence during Legal Investigations" & "General Guidelines for the Collection and Recording of Entomological Evidence". Other sources (Lord & Burger 1983; Catts & Haskell 1990; Wecht 1995) can also be consulted for insight into detailed instructions.

1. *Crime Scene Approach*

When using entomological evidence in legal investigations it becomes important to record the physical surroundings of the crime scene before collection of evidence occurs. Such records include detailed notes and photographs of the environment in which the crime was committed as well as meteorological data. The collection of such information provides an initial assessment of the crime which can be collaborated with sampled entomological evidence.

As the collection of entomological evidence can be somewhat intrusive, in regards to extent of collection and the location of evidence, it is practice to await permission from the crime scene manager before entering the crime scene to collect evidence. In most cases the coroner/medical examiner maintains jurisdiction over the body at a crime scene, and permission must be granted by them before collection of evidence from the body takes place. Before proceeding with collection from the body, indications of where sampling will occur must be explained to the coroner or medical examiner present. This will limit the impact of disturbance, caused by sampling, in the coroner's report. Once initial collection of entomological evidence is complete from the body and its immediate vicinity, further surface sampling from beneath the body and surrounding areas may be conducted once the body is removed. Later sampling can also be conducted during the autopsy procedure under the guidance of the medical examiner. In most cases, the forensic entomologist does not perform

collection of evidence themselves and it is instead commonly collected by trained crime scene personnel. This highlights the need for training of crime scene personnel in the proper collection procedure of entomological evidence.

Coordination is a key element during any crime scene investigation to limit disturbance and loss of evidence. The forensic entomologist must be thoroughly briefed on the circumstances that the body was discovered and any information as to the alteration of the crime scene and already sampled evidence before their arrival. Similarly the forensic entomologist must provide any information, regarding sampling methods, collected specimens and general information obtained, to the crime scene personnel. As no two crime scenes are the same there is no set sequence of events that must be followed when collecting entomological evidence, but collection of primary evidence takes precedence. The process of collecting entomological evidence often takes place together with the collection of other physical evidence and will depend on the circumstances of death, environmental factors and type of evidence present.

2. *Collection of Entomological Evidence*

During the actual process of entomological evidence collection, it becomes important to limit disturbance to the crime scene as much as possible while attempting to gain sufficient samples. Slow movements while in the crime scene minimize arthropod faunal disturbance, particularly to flying adults, and provides opportunities to make observations and acquire photographic evidence. These initial observations allow investigators to determine where the main areas of colonisation occurred on a body, as well as the current stage of decomposition the body was in. Photographic evidence also provides information that may have been missed during evidence collection. During these observations it becomes important to note migration activity of arthropod fauna from the body as this will indicate whether surface sampling is necessary. Once preliminary observations have been made, collection of samples can then proceed once permission is given.

Byrd *et al*. (2010) broke entomological documentation and collection of samples down into several major steps as follows:

1. Photograph and obtain written notes of the general characteristics and habitat of the crime scene

2. Photograph and obtain written notes on the state of arthropod colonisation on and around the body
3. Collect meteorological data from the crime scene
4. Collect adult arthropod individuals on and around the body
5. Collect egg clusters, larvae and pupae (when present) on the body
6. Collect specimens from the surrounding area surface, up to 6m away from the body
7. Once the body has been removed from the crime scene, collect specimens from directly beneath and within the immediate vicinity of the where the body was (if present)
8. Collect soil samples from where the body was (depending on surface type)
9. Make notes as to the ecological characteristics of the crime scene, pertaining to soil, plant cover, water etc. (if indoors, make notes on the state of the room regarding open doors/windows, cleanliness, floor type etc.)
10. Collect further entomological samples from body during autopsy
11. Obtain historical climate/weather data from nearest weather station to the crime scene

The evidence collected is often recorded on specific forms to allow for simpler filing and retrieval. Many of the collected specimens are bred to the adult stage to allow for easier identification while some of the dipteran larvae are preserved in order to measure their length and instar stage. These results combined with historical weather data allow for estimation of the Period of Insect Activity (PIA) and in certain cases PMI. As new procedures are being developed, more recent collection methodology must be consulted in order to orientate oneself with current developments in this field.

While being an arduous task, the collection of evidence provides the greatest insight into a crime, and the information obtained during entomological evidence sampling has been found to reveal evidence that was overlooked (Benecke 2004). Any piece of evidence can prove invaluable to an investigation, and the role of forensic entomology is only a small part of a much larger collaboration in legal investigations.

4.4.2 Suggested Excavation and Collection of Entomological Evidence Methodology from Clandestine Graves

While the previously mentioned methods of evidence collection can be adapted to various crime scene situations, certain methods become difficult to perform under specific conditions. Burial situations prevent ease of access for collection of evidence, and problems are encountered whereby excavations with heavy equipment on deeply buried bodies can cause extensive damage to the remains. Furthermore, arthropod faunal activity and behaviour differs from above-ground, with specific regions being colonised and difficulties in collecting adult individuals. As such, alternative methods are required when attempting to collect physical evidence from buried remains. This section provides suggestions to simplify excavation methods and collection of entomological evidence from clandestine graves in central South Africa during dry winter and wet summer seasons.

1 *Excavation Methodology*

When attempting to recover evidence from a burial site it becomes essential to record the process of recovery so that the scene can be reconstructed and the guidelines, as to how recovery was performed, is available (Walsh-Haney *et al*. 2010). During the recovery process from a crime scene, all information pertaining specifically to the body and its position cannot be physically recreated, thus it becomes imperative to record all available information from the start of recovery (Skinner & Lazenby 1983). While physical evidence is often the most sought after at a crime scene, non-physical evidence can be just as important, particularly in regards to the depth of the body, its position, location, physical appearance and orientation. It is therefore important to record all characteristics of the body during excavation. The brief guidelines stipulated here are those suggested by Walsh-Haney *et al*. (2010).

Once located, gridlines must be placed over the gravesite to orientate investigators as to location of evidence and mapping of the grave. Photographs become a prominent feature during burial excavations to record the characteristics of the surrounding area and of the grave itself. A small group of 3 to 4 people is required in most cases as an excessive number of workers may disturb the site and compromise evidence. When the area is mapped out, and excavation begins, any photographs that are taken must include a reference point such as a depth stick and a north arrow. All changes in soil composition and tool use should be noted

and, if possible, photographed. Notes on the conditions of the grave, in regards to its size, vegetation, signs of disturbance, arthropod activity and decomposition, must be made. It is advisable that during crime scene excavation a trained archaeologist, or member of the team with experience in burial recovery, be present. The edges of a grave can often be discovered using a simple digging tool. The soil on the outer rim of the grave is often compact and denser than the soil directly above the body which has become loose due to burial (Pickering & Bachman 1997). The mapping of these initial edges will help excavators when they begin to dig.

Excavation processes often depend upon the estimated depth of the burial and soil conditions. In certain cases, such as waterlogged graves, drainage is required before excavation may begin. Soil is removed from the grave in layers of 10 cm, with samples of the soil taken at regular intervals for screening of evidence. Detailed notes of soil removal must be maintained to indicate at which depths abnormalities and evidence were found and recovered. To avoid contamination, loose soil is completely removed from the grave and, in instances of disturbance by scavengers, the soil is screened for evidence. Depending on the size of the grave, soil from the edges may be too loose to allow excavators to lean on. In these cases it is advisable that a parallel pit be dug next to the grave to allow excavators to work in safety at a similar level to the body. Although the presence of this pit will destroy one side of the grave, the original edge must be traced and recorded as depth increases. Once the body is reached, smaller tools are necessary to carefully remove soil around the remains and soft bristled brushes are used to expose features. Brushing must always be done in one direction as back and forth motions will obscure soil changes and dislodge evidence. During cases where the remains are not skeletonised, gentle scraping of the soil from the body is conducted to prevent damage. Proper archaeological procedures advise that it is more useful to expose only half of the grave in order to establish a profile. However, in forensic cases, due to the relatively small size of graves often encountered by investigators, such procedures cannot always be followed. Screening of the soil removed from graves often yields the most evidence as entomological specimens, bones and physical evidence are easily overlooked during excavation.

When exposing the body, care must be taken to prevent damage to the remains and hastily removing soil is not advisable. Mapping of the remains is done while the body is exposed, and this mapping is three dimensional to determine the true position within the grave based on the grid lines and depth. Positions of the body and appendages are recorded using this

three dimensional mapping. When the remains are fully exposed, photographs become essential before the body is removed from the grave. Further notes must be taken in regards to the body's relation to its surroundings in the grave.

After the removal of the body, final checks are conducted to ensure all aspects of the scene are recorded and mapped. Depending on the depth of the grave, soil samples may be taken from beneath the body's location to screen for entomological evidence, particularly in shallow graves. Further sampling of evidence is then performed during the autopsy. Lastly, a final profile of the grave is made in regards to the actual depth of the grave within the centre of the pit.

The brief guidelines stated above are usable for excavations within central South Africa. However, certain abnormalities were noted that may impact these guidelines and alternative methods are suggested.

During the excavation of the winter and summer graves, the soil was noted to be easily removed from the graves and the edges of the graves were easily located due to the looseness of the soil. However, the edges were particularly susceptible to cave-ins during excavations. It is therefore advisable that parallel pits be dug during burial cases in central South Africa. The soil type of each grave differed at 60 cm depths and, during winter, large clumps of soil were present throughout the graves. These clumps can hamper proper soil layer removal. It is suggested that the clumps of soil be removed individually when found and placed separately for analysis during excavation to prevent problems with soil screening. Due to the heavy rainfall experienced during the summer trial, the soil of the graves became waterlogged throughout and layer excavation became nearly impossible. In these instances, as previously stated, drainage of the soil is needed. Waterlogged soil that is removed from such graves must be dried before screening. When the bodies were reached, large amounts of fluid seepage was seen beneath the bodies and skin slippage occurred as decomposition progressed. When excavating within central South Africa it is advisable that the body be removed from the grave together with the bottom layer of soil to prevent loss of contextual evidence.

These suggestions may help investigators to limit disturbance of buried remains and prevent loss of evidence during collection and excavation within central South Africa.

2. *Entomological Evidence Collection Methodology*

Byrd *et al*. (2010) stated that the collection of entomological evidence during a burial crime scene will follow similar guidelines to those previously mentioned. However, Walsh-Haney *et al*. (2010) stipulated that these guidelines may have to be altered when dealing with buried remains. Due to decomposition and colonisation occurring at a slower rate below-ground, changes in faunal activity have been reported (Lundt 1964; Payne *et al*. 1968). In some cases unique faunal activity has been found to occur on buried remains (Leclercq 1975).

While collection of entomological evidence occurs primarily from the body in above-ground crime scenes, soil screening often yields a number of samples in burial cases. Based on the findings of the present study, recommendations are suggested regarding collection of entomological evidence.

With colonisation being reduced to only a few species and a lesser abundance, compared to above-ground, care must be taken to obtain sufficient evidence. Some insect species, such as members of the Phoridae, were able to colonise buried remains as adults, while other species (*Muscina stabulans* and the Sarcophagid sp.) oviposited within the top layers of the grave soil. These larvae then burrowed down to the remains, indicating the importance of screening soil samples taken during excavations. With different colonising methods being utilised by arthropods, no single collection method can be used during crime scene excavations. This will cause delays in collecting evidence as investigators will continually be excavating into the unknown. It therefore becomes important for researchers to ascertain what species may be present on buried remains in certain geographical locations to enable easier collection during burial investigations.

While the collection guidelines stipulated by Byrd *et al*. (2010) can be generally utilised during burial investigations in central South Africa, a number of problems were noted during this study pertaining to collection efficiency. Alternative methods are therefore suggested.

During the winter trial, large numbers of phorid adults were seen to colonise buried carcasses by day 21 of the trial. Once the body was reached during excavation, and loose soil removed from around it, groups of these phorid adults immediately escaped from the grave when exposed to open air. Similarly, adult *Leptocera* sp. individuals escaped from grave six (day 60) during the winter trial in groups once disturbed and exposed to air. The sudden migration of these phorid adults was unexpected, and difficulty was experienced in attempting to collect

individuals due to a lack of preparedness. In most crime scenes, entomological samples are often not considered primary evidence and are collected together with other physical evidence. However, the sudden migration of entomological data during excavations may cause a loss of evidence for forensic entomologists. Even though photographs must be extensively taken during crime scene investigations, live samples provide a much more detailed medium for identification, especially in the light of the relatively small size of the members of Phoridae. Identification of sampled dipteran specimens from buried carcasses was the main focus of the parallel study by van der Merwe (2016 *unpublished*), which provides a morphological key to identify the Diptera currently known to colonise buried carcasses in central South Africa. Consideration must therefore be put towards preparedness of sampling adult insect specimens before they escape and evidence is lost. This may mean a shift in categorising entomological evidence as a form of primary evidence during excavations. This shift may also bring questions as to the risk of disturbance to the grave during sampling of these specimens, as is explained later on.

Heavy rainfall caused all graves to become waterlogged during the summer trial. Drainage of these graves would prove difficult, particularly due to the high clay concentration in many of the grave soils. Sarcophagid pupae were present within the first 30 cm of grave six (day 60) of the trial, while larvae were found in the disturbed grave one between 30 cm to 60 cm depth. This reinforces the use of the previously mentioned guideline to remove soil in 10 cm layers and screen these samples to attain evidence. Soil removed from these waterlogged graves must first be dried before screening is attempted.

Niche partitioning by phorid adults, larvae and pupae were recorded to occur in specific regions of the carcasses, specifically the stomach, legs and sections underneath the carcasses. When sampled, parts of the carcasses needed to be removed in order to reach the immatures. This invasive sampling caused artefacts and disturbance on the carcasses. While such sampling is often conducted during the autopsy of the body under the jurisdiction of a medical examiner, the presence of predatory staphylinid *Aleochara* sp. adults and larvae during the summer season becomes a problem. If the body was to be removed from the grave for an autopsy analysis, while predatory arthropods remained on it, it may lead to a loss of entomological evidence due to predation. It therefore becomes necessary to determine when proper sampling of entomological specimens can be performed on the body to attain sufficient evidence, depending on the abundance of predatory species. During instances

whereby sampling must be done at the crime scene to prevent loss of evidence, coordination with the medical examiner becomes vital.

The mite *Sancassania mycophagus* was seen to also colonise the stomach of buried carcasses, with extensive overlaps occurring with predatory staphylinid *Aleochara* sp. adults and larvae. By taking cognisance of this niche partitioning, as observed during the present study, investigators may be able to lessen collection times in the field and during autopsies within central South Africa.

These suggestions are given to allow for ease of collecting entomological evidence within central South Africa, and points out the importance to know what species occur during each season and the possible trophic interactions that may compromise entomological evidence. It is recommended that investigators take note of the differences of burial environments compared to above-ground crime scenes.

CHAPTER 5

Concluding Remarks

CONCLUDING REMARKS

5.1 Summary of Arthropod Succession, Decomposition and Biomass Loss Results

With the aims of this study determining the impact of burial on arthropod succession, decomposition and biomass loss, conclusions as to the results obtained can be made.

1. *Arthropod Succession*

Overall, arthropod succession remained faster and more abundant above-ground during both seasons with burial influencing more selective succession and colonisation. From the results obtained it can be predicted that initial colonisation of buried carcasses, during typical seasons, occurs within 14 to 21 days after burial within central South Africa, with Diptera playing a prominent role. Further succession waves can occur 30 days after burial during winter and summer, leading to possible further usage of time frames to determine PBI.

While succession and colonisation of carcasses occurred at a much faster rate above-ground, more selective colonisation occurred on buried carcasses. With blow fly and flesh fly species dominating above-ground, as is often the case, the phorid species *Megaselia scalaris* and *Conicera tibialis* dominated buried carrion during the winter season. Heavy rainfall during the summer culminated in lower dipteran abundance below-ground, with only *M. scalaris* adults occurring on the buried carcasses. The presence of *Muscina stabulans* and a sarcophagid species below-ground near the end of the winter and summer trials respectively, indicates ability of more robust flies to colonise buried carrion, supporting the potential of utilising select arthropods in PBI estimations. The presence of a *Leptocera* species on buried carcasses during winter also indicates the possibility of indicators being of importance during investigations. However, due to the feeding habits of this species being unknown it cannot yet be determined what impact it may have on estimations when fungal growth is present.

Abundance of *Dermestes maculatus* immatures at differing larval stages on the above-ground winter carcass indicates potential usage of this species during winter months in PMI determination. However, further investigations are required to determine the exact viability of such a species. With climate change becoming an ever present factor, temperatures and seasons are expected to fluctuate more often which may lead to certain species abundance and dominance changing during seasons.

The mite *Sancassania mycophagus* was seen to colonise the buried summer carcasses in clusters on the stomach and legs. The role of this species in forensic burial cases is unknown at present as this species is often found on stored products and asparagus plant roots. More studies are therefore required in order to determine the exact role of this species in burial cases, as other mite species have been found to play a role in previous forensic cases.

The results obtained supported the hypothesis that arthropods will be able to colonise buried cadavers at 60 cm. However, the hypotheses that faster colonisation and a higher

abundance and diversity will occur during summer on buried cadavers was proved incorrect. This was due to the high impact of rainfall and soil composition on the graves preventing increased successional rates during summer. Additionally the hypothesis of Coleoptera species playing a significant role in below-ground succession was partly correct. Coleoptera species did not play any significant role during the winter season below-ground, but its significance during summer was noted with the presence of a staphylinid *Aleochara* species. Adults of this species were seen to prey upon dipteran phorid adults and mites of the species *S. mycophagus*. Unfortunately, as stated by previous studies, whether such coleopteran species are of forensic importance, and whether they are reliable in PBI determination, remains to be seen due to their ecological relationships. Despite this, predatory coleopteran species may have an impact on arthropod succession in burial cases depending on the species present and their abundance.

2. *Decomposition and Biomass Loss*

Temperature and rainfall were the main factors influencing decomposition and biomass loss rates during the winter and summer trials. With typically slower rates of decomposition on the above-ground winter carcass, due to low ambient temperatures, less biomass was lost. Of interesting note was the partial mummification of the above-ground carcass due to relatively low humidity and cold winds. Buried carcasses followed a faster rate of decomposition, and thus biomass loss, compared to above-ground in winter due to isolated environments limiting fluctuation of temperatures as well as providing shelter to select arthropods that were able to colonise the bodies. This in turn had an effect on bacterial and enzymatic activity within the graves. Fungal growth became an every present factor on buried carcasses causing possible attraction of dipteran indicator species. An increased rate of decomposition, both above- and below-ground, was observed during summer months compared to winter. However, decomposition was slower on buried carcasses during this season. Heavy rainfall, causing waterlogging of the graves, increased decomposition rates of buried carcasses during summer compared to winter as anaerobic bacterial activity became more prominent.

These results, once again, partly supported the hypotheses of biomass loss and decomposition. While decomposition was slower below-ground, and greater biomass was lost above-ground during the summer season, winter results showed the complete opposite. Therefore, it can be established that higher rates of decomposition and biomass loss will occur on buried cadavers, compared to above-ground, during winter seasons due to insulation provided by the soil, promoting bacterial and fungal activity at a narrow temperature range. Summer months, however, will see a decrease in these rates below-ground during higher rainfall with the insulating property of the soil preventing higher temperature ranges compared to above-ground.

Soil type becomes an important factor to take into consideration when determining decomposition as clay based soils were seen to drastically increase putrefaction and adipocere formation on buried carcasses. Furthermore, characteristics utilised to determine the stage of decomposition were seen to deviate from characteristics seen on the buried carcasses. This included lack of inflation of buried carcasses during bloated stage as well

as increased putrefaction and fungal growth during active decay. This highlights the importance of establishing adequate decomposition characteristics in regards to burial.

While the results obtained from this study show the ability of select arthropods to colonise buried cadavers, as well as differences in decomposition and biomass loss rates, during summer and winter, more research is required before an adequate picture of succession and decomposition, applicable to South Africa, is obtained. Variations within differing geological, ecological and seasonal circumstances will become the key factor in burial cases, providing investigators with the tools to determine PBI. Predicted

5.2 Alternative Suggestions for Collection and Excavation Methodology

During the winter and summer trials, circumstances regarding collection and excavation methodology were noted to differ in regards to established guidelines. Suggestions and alternatives to these guidelines are given for the central South African region.

1. *Excavation Methodology*

   During the winter and summer excavations, soil removal was noted be relatively simple with the edges of the grave being easily located due to differences in looseness and compaction of the soil. While the looseness of the soil allowed easy removal from the graves under dry conditions in winter, the opposite was seen during the summer trial as heavy rainfall had caused subsequent waterlogging of the graves. Such waterlogging prevented layered removal of the soil and, in this regard, drainage of the graves is required in order to effectively excavate the remains. Soil that is removed from such waterlogged graves must be dried before it is screened. Large clumps of soil were also found while excavating the dry winter graves, preventing simple layered removal of soil in certain cases. It is suggested that individual removal of such clumps be made during excavations and these clumps be stored and screened separately.

   Despite the easily located borders of the graves from both seasons, during excavations the edges were found to be susceptible to cave-ins in both dry and waterlogged conditions. Such cave-ins compromise evidence and it is suggested that parallel pits be dug during crime scene excavations in central South Africa to prevent loss of evidence in burial cases. It was also noted during the seasonal excavations that the carcasses of the graves released large amounts of decomposition fluids beneath the body and skin slippage became prominent. This caused parts of the carcass to remain attached to the soil beneath the body if the carcass was forcibly removed. It is therefore suggested that the underlying soil layer of the grave be removed in conjunction with the carcass to prevent loss of contextual evidence.

2. *Collection Methodology*

   As excavations took place during the winter and summer trials, specific colonisation by arthropods was seen, this led to certain areas of the carcasses being colonised as the trials progressed. While entomological evidence is often collected conjointly with other physical

evidence, an occurrence during the excavations brought the reliability of this process into question. When the winter buried carcasses from day 21 onwards were reached, and the soil surrounding the bodies was removed, large numbers of phorid adults escaped from the grave when exposed to air. This sudden migration was unexpected, and difficulty was faced attempting to sample these adult individuals due to a lack of preparedness. While entomological samples are often not considered primary evidence, the possible sudden loss of such arthropod species during excavation must be taken into consideration at a crime scene. It is suggested that crime scene investigators ensure preparedness during burial cases to prevent loss of evidence, as live samples provide a more reliable medium for identification compared to photographs, particularly in regards to the size of the members of Phoridae.

Niche partitioning was evident during the winter and summer trials as the arthropod species that were able to colonise the buried carcasses were sampled from specific regions. This niche partitioning may prove beneficial to crime scene investigators to lessen collection times during an investigation. Despite these specific regions of the body being colonised, it was noted that sampling of dipteran larvae and pupae on the buried carcasses became difficult due to their size. This was especially true for the phorid species as many of the larvae and pupae were found underneath the skin and soil present on the carcass. In order to sample these immatures, the skin and soil would have to be removed. However, this can damage the remains and cause a loss of evidence. It is therefore suggested that sampling of entomological evidence from the body be delayed until it is removed and the autopsy is performed.

Another difficulty with this situation is the presence of predatory arthropod species on the buried carcass. Staphylinid adults present on the summer buried carcasses were seen to prey upon only adult phorids as well as mites colonising the carcasses. This predatory behaviour can cause errors in PBI estimations as individuals that were the first to colonise buried remains may be predated upon causing incorrect estimations to be made. In the instance that buried remains must be removed before sampling of entomological evidence can take place, the concern that a loss of evidence may occur becomes a factor. In this regard coordination with the presiding medical examiner becomes crucial if the risk of losing entomological evidence becomes apparent. Decisions must then be made regarding initial sampling from the buried remains at the site while attempting to prevent disturbance on the body or possibly lose evidence before sampling can be conducted during the autopsy procedure.

While the results obtained from the excavations of this study show alterations and differences to current guidelines, these suggested alternatives are most applicable to the conditions experienced during the winter and summer trials of this study, in central South Africa. As with all crime scene investigations, coordination between crime scene personnel becomes a crucial factor in order to limit disturbance and loss of evidence.

CHAPTER 6

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APPENDICES

APPENDIX A
Ethical approval

The following ethical approval form was received for the present study:

"**Decomposition and arthropod succession on buried remains during winter and summer in central South Africa: Forensic implications and predictive analyses**"

The application was accepted under the Animal Experiment Number: 24/2014, by the University of the Free State Animal Ethics Committee.

The project title in the application form was changed from:

Jason Botham: "A comparison of above ground and below ground arthropod succession, with special focus on Coleoptera and decomposition"

to the present approved title in January 2016.

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Me / Ms H Viljoen

2014-10-28

DR SL BRINK
DEPARTMENT OF ZOOLOGY & ENTOMOLOGY
D206 C
FACULTY OF NATURAL AND AGRICULTURAL SCIENCES
UFS

Dear Dr Brink

ANIMAL EXPERIMENT NR 24/2014
RESEARCHER: DR SONJA L BRINK - DEPARTMENT OF ZOOLOGY & ENTOMOLOGY
PROJECT TITLE: (1) SYLVIA VAN DER MERWE: "AN ANALYSIS OF SEASONALITY AND DEPTH ON THE OCCURRENCE OF DIPTERAN SPECIMENS IN BURIAL CASES, WITH SPECIAL FOCUS ON PHORIDAE"
(2) JASON BOTHAM: "A COMPARISON OF ABOVE GROUND AND BELOW GROUND ARTHROPOD SUCCESSION, WITH SPECIAL FOCUS ON COLEOPTERA AND DECOMPOSITION"

You are hereby kindly informed that the Interfaculty Animal Ethics Committee approved the above study and it will be condoned at the meeting scheduled for 30 October 2014.

ANIMAL	*NUMBER*	*EXPIRY DATE*
Pigs	7	April 2016

Kindly take note of the following:

1. ***Fully completed and signed applications have to be submitted electronically to viljoenjj@ufs.ac.za and a hard copy has to be submitted too.***
2. ***A signed progress report with regard to the above study has to be submitted electronically to viljoenjj@ufs.ac.za while a hard copy has to be submitted to Ms H Viljoen, Room D115, Francois Retief building, Faculty of Health Sciences. A report has to be submitted when animals are physically involved and after completion of the study. Guidelines with regard to progress reports are available from the secretary and on the Faculty Intranet.***
3. ***Researchers that plan to make use of the Animal Experimentation Unit must ensure to request and receive a quotation from the Head, Mr Seb Lamprecht. A copy of the quotation has to be submitted with the application before the application will be considered for approval.***
4. ***Fifty (50%) of the quoted amount is payable when you receive the letter of approval.***

Regards

.....
CHAIR:
INTERFACULTY ANIMAL ETHICS COMMITTEE

APPENDIX B

B1.1 General Above-ground Ecology of *Dermestes maculatus*

The commonly known hide beetle, *Dermestes maculatus*, has been known to be of forensic importance in homicide cases (Richardson & Goff 2001). These beetles usually reside around resources where adult beetles will feed and eventually mate over time, typically attracted by strong pheromones secreted by males (Rakowski & Cymborowski 1986). Although aggregation of the species may vary in size according to the size of a food source (McNamara *et al.* 2008), the species has been found to dominate in some cases and compete strongly for a food source (Odeyemi 1997).

The intensity at which *D. maculatus* will compete for a food source is dependent on the conditions which the food source is exposed to, but opportunism behaviour in unfavourable conditions is an adaptation that many insects adopt in order to survive (Ives 1988; Turchetto & Vanin 2004; Stephen *et al*. 1969). The winter trial of this study may have strongly highlighted this behavioural change, showing dominance of *D. maculatus* over a sixty day period on an above ground carcass. To fully understand the movement of a species such as *D. maculatus* on a food source, observations were made daily after their arrival, and significant changes in activity and abundance were documented (Table B1).

Table B1: Recordings and descriptions of *Dermestes maculatus* abundance, breeding and general ecology on the 2014 winter control carcass from day 14 (05/07/2014) onwards.

Day	Abundance	Description
14	10 – 20 adults	First occurrence of *D. maculatus* adults on control carcass.
15	10 – 20 adults	Adult *D. maculatus* individuals seen roaming around carcass.
16	10 – 20 adults	Adults continue to roam body, particularly around head of carcass. *Lucilia* sp. adults seen around head and mouth of carcass.
17	10 – 20 adults	Large number of adults in mouth of carcass feeding on eggs recently oviposited by *Lucilia* species.
18	10 – 20 adults	Adults continue to congregate around mouth and feed on unhatched dipteran eggs.

Table B1: Continued.

Day	Abundance	Description
19	10 – 20 adults	Group of *D. maculatus* adults still remaining around mouth and head of carcass.
20	10 – 20 adults	Decrease in number of adults around mouth and head noted, larger number roaming on body.
21	10 – 20 adults	Adult *D. maculatus* individuals continually roaming on body. Calliphoridae species seen mating on cage.
22	10 – 20 adults	Calliphoridae species seen ovipositing beneath forearm of carcass with *D. maculatus* adults roaming near and a single adult feeding immediately on oviposited dipteran eggs (Figure B1).
23	< 5 adults	Decrease in *D. maculatus* abundance noted. Possible competitive action between species?
24	7 – 15 adults	Adult *D. maculatus* individuals seen feeding on cluster of newly oviposited *Lucilia* sp. eggs underneath forearm of carcass.
25	10 – 15 adults	Large cluster of adults in left nostril of carcass, presumably feeding on dipteran eggs. Single sarcophagid sp. adult seen underneath forearm of carcass.
26	10 – 20 adults	Adults clustered in left ear of carcass feeding on dipteran eggs (Figure B2).
27	10 – 20 adults	Continued feeding by adults in left ear; few adults observed searching around the body.
28	10 – 20 adults	Continued feeding by adults in left ear; adults seen fending off few dipteran species around the body.
29	10 – 20 adults	Migration of adults from ear to below the body and to mouth area (possible feeding in the mouth).
30	None present	No adults seen on body, coincides with sarcophagid sp. larvae.

Table B1: Continued.

Day	Abundance	Description
31	10 – 20 adults	Small number of adults noted occurring on carcass for the remainder of the day despite presence of Sarcophagid sp. immatures.
32	None present	No adults seen on body, coincides with Sarcophagid sp. larvae.
33	None present	No adults seen on body, coincides with Sarcophagid sp. larvae.
34	15 – 20 adults	Sudden reappearance of adults on carcass; Possible recolonisation for the purpose of reproduction.
35	15 – 20 adults	Adults seen concentrated near the underside of the body; Possible recolonisation for the purpose of reproduction.
36	15 – 20 adults	Adults seen concentrated near the underside of the body; Possible recolonisation for the purpose of reproduction.
37	15 – 20 adults 10 – 20 larvae	First occurrence of *D. maculatus* larvae on body (Figure B3).
38	15 – 20 adults 10 – 20 larvae	*Dermestes maculatus* larvae seen roaming on body, particularly on stomach of carcass.
39	15 – 20 adults 10 - 20 larvae	Larvae of *D. maculatus* concentrated below the body and underneath folds of skin on the carcass.
40	15 – 20 adults 10 - 20 larvae	Adult *D. maculatus* seen around the legs and abdominal area as well as below the body; larvae of *D. maculatus* concentrated below the body and underneath folds of skin on the carcass.
41	15 – 20 adults 30 – 40 larvae	Increase in abundance of *D. maculatus* larvae seen on carcass.
42	15 – 20 adults 30 – 40 larvae	Larvae and adults seen to be concentrated underneath the body; some *D. maculatus* adults seen around the folds of the legs and around left ear.

Table B1: Continued.

Day	Abundance	Description
43	15 – 20 adults 50 – 60 larvae	*Dermestes maculatus* larvae present in ear of carcass, further increase in abundance seen.
44	15 – 20 adults 50 – 60 larvae	New holes in carcass skin discovered below the left arm; some *D. maculatus* larvae seen burrowing into holes (Holes possibly left behind by Sarcophagidae larvae as artifacts).
45	15 – 20 adults 60 – 70 larvae	Generally scattered movement of *D. maculatus* adults observed below the body; larvae seen migrating to ears and mouth.
46	15 – 20 adults 60 – 70 larvae	Aggregation of *D. maculatus* adults around mouth and ears of carcass.
47	15 – 20 adults 70 – 80 larvae	Gradual increase in larval *D. maculatus* specimens on body – possibly a sign of continued breeding on the body.
48	15 – 20 adults 70 – 80 larvae	Adult *D. maculatus* seen competing with *Necrobia rufipes* – competition and coexistence; Larvae seen around legs and mouth area.
49	15 – 20 adults 80 – 90 larvae	Adult *D. maculatus* seen competing with *N. rufipes* – competition and coexistence; gradual increase in larval *D. maculatus* specimens on body – possibly a sign of continued breeding on the body.
50	15 – 20 adults 80 – 90 larvae	Larvae in mouth of carcass. Possible feeding occurring.
51	15 – 20 adults > 100 larvae	Spike in *D. maculatus* larvae number of individuals noted; some adults seen moving away from the carcass, adults mainly concentrated below the body.
52	15 – 20 adults > 100 larvae	Larvae underneath arm of carcass; adults mainly concentrated below the body.
53	15 – 20 adults 80 – 90 larvae	Various *D. maculatus* larval stages on body (Figure B4), possible feeding holes seen on stomach of carcass (Figure B5).

Table B1: Continued.

Day	Abundance	Description
54	15 – 20 adults 80 – 90 larvae	Later larval instars of *D.maculatus* immatures aggregating underneath body.
55	15 – 20 adults 70 – 80 larvae	Previous casings of larvae seen beneath carcass indicating molting and growth had occurred.
56	15 – 20 adults 70 – 80 larvae	Some adults seen moving away from the carcass, adults mainly concentrated below the body; Larvae generally large and highly active below the body (Figure B6).
57	15 – 20 adults 70 – 80 larvae	Adults migrating away from food source – intraspecific competition causing need to seek new food source.
58	15 – 20 adults 70 – 80 larvae	Adults migrating away from food source – intraspecific competition causing need to seek new food source.
59	15 – 20 adults 65 – 75 larvae	Slight decrease in abundance of *D. maculatus* larvae seen; Adults migrating away from food source – intraspecific competition causing need to seek new food source.
60	15 – 20 adults 65 – 75 larvae	*D. maculatus* larvae still in high abundance on the control carcass (Figure B7). Larvae seen entering carcass through openings and definite feeding observed (Figure B8).

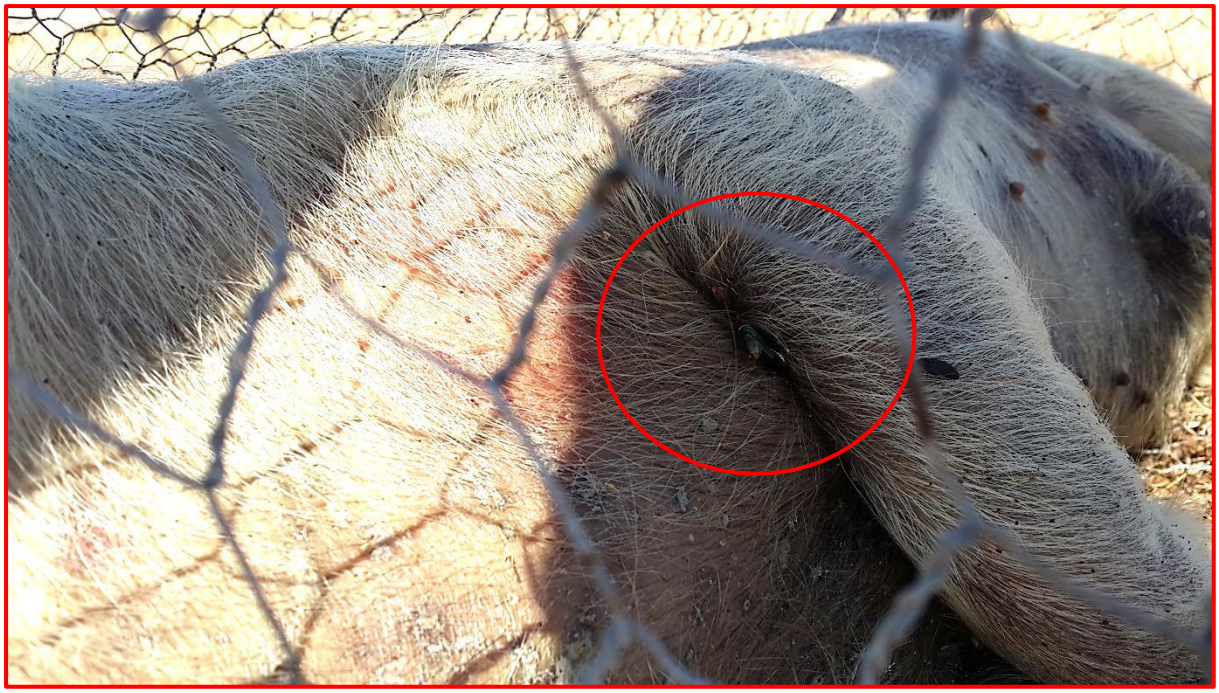

Figure B1: Adult *Dermestes maculatus* feeding on oviposited dipteran eggs beneath forearm of winter control carcass (day 22, 13/07/2014).

Figure B2: Cluster of *Dermestes maculatus* adults feeding on oviposited dipteran eggs in ear of winter control carcass (day 26, 17/07/2014).

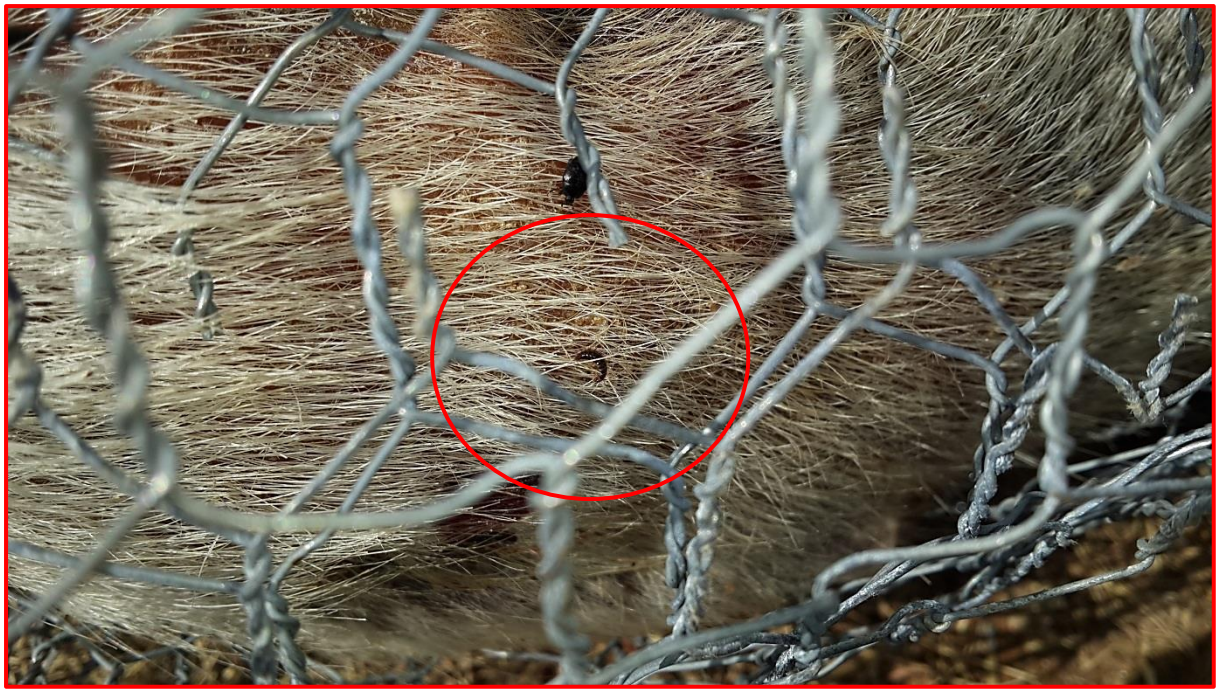

Figure B3: One of the first *Dermestes maculatus* larvae seen on the winter control carcass (day 37, 28/07/2014).

Figure B4: Various *Dermestes maculatus* larval stages present on winter control carcass (day 53, 13/08/2014).

Figure B5: Evidence of possible feeding by *Dermestes maculatus* larvae on winter control carcass (day 53, 13/08/2014).

Figure B6: Large *Dermestes maculatus* larva found below the body of the winter cadaver (day 56, 16/08/2014).

Figure B7: High abundance of *Dermestes maculatus* larvae on winter control carcass (day 60, 20/08/2014).

Figure B8: Evidence of *Dermestes maculatus* entering the winter control carcass and feeding (day 60, 20/08/2014).

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Oral Presentation: Sylvia S. van der Merwe, Jason L. Botham, Sonja L. Brink. An Analysis on the Effects of Seasonality on Arthropod Succession and Decomposition of Buried Cadavers in a Central Free State Grassland Area.