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INSECT COLONISATION OF BODIES BROUGHT INTO THE JOHANNESBURG FORENSIC PATHOLOGY SERVICE: IMPLICATIONS FOR ACCURATE TIME OF DEATH ESTIMATIONS



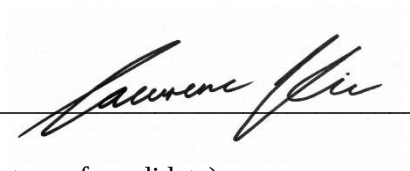
Lawrence Hill

A dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, in fulfilment of the requirements for the degree of Master of Science in Medicine

Johannesburg, 2019

DECLARATION

I, Lawrence Hill, declare that this Dissertation is my own, unaided work. It is being submitted for the Degree of MSc (Med) at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.



(Signature of candidate)

_____**22**_____day of____**February**_____20**19**___in__**Johannesburg**__

DEDICATION

In loving memory of my grandfather

Arthur Marco Jooste

1939 – 2014

In loving memory of my grandmother

Hendrika Jacoba Hill

1926 – 2017

PUBLICATIONS ARISING FROM THIS STUDY

Hill, L., Gilbert, A.E., Coetzee, M. 2018. Modelling Temperature Variations from the Death Scene to the Time of Insect Collection at Autopsy: Implications for Estimation of the Post-Mortem Interval. *Journal of Forensic Science* (**In Preparation**).

ABSTRACT

Forensic entomology involves the analysis of insects that colonise human bodies after death. These insects become associated with the body shortly after death, in a continual succession as decomposition progresses. This dissertation provides an analysis of the insect taxa associated with human bodies and factors that may affect the species and number of taxa on the remains. This dissertation also provides a simulated model of the post-mortem interval (PMI) estimation, using the Monte Carlo simulation. Published base temperature measures for six dipteran species collected after autopsy, were used to estimate variations in PMI. A total of 33 bodies, out of 3,427 (0.96%) received by the Johannesburg Forensic Pathology Service for post-mortem examination over a 16-month period, were colonised by insects. No cause of death was determined in 48.48% of the colonised bodies as a result of the degree of decomposition. *Calliphora vicina* was the most commonly occurring species followed by *Chrysomya albiceps, Ch. chloropyga* and *Lucilia sericata*. Bodies in the active decay stage were found to have the highest species richness with nine insect taxa found. Logistic regressions were performed to determine the factors affecting the number of taxa present and the species occurring on the remains, and both temperature and the stage of decomposition were found to be significant. The importance of temperature in this study is further reiterated in the Monte Carlo simulation using the modelled accumulated degree days from the time the body was removed from the death scene, refrigerated and finally autopsied, before insect collections were performed. Six species of Diptera were modelled, including *Calliphora vicina, Chrysomya albiceps, Ch. chloropyga, Lucilia sericata, Musca domestica* and *Piophilia casei*. Temperature measurements recorded over the duration of the first half of the study were used as input variables in the simulation. From the simulation it was found that temperature fluctuations could result in continued development of insect larvae during refrigeration. However, all species except *Ca. vicina* were found to have less than a 45% likelihood of continued development, while *Ca. vicina* was found to have 76% likelihood of continued development during refrigeration. This continued development was found to produce increased mean PMI estimations of between 11.45 and 13.37 accumulated degree-days (ADD) with standard deviations of 6.79 to 7.42 ADD depending on the species. These results highlight the importance of temperature for the taxa present, the number of taxa, and the ability to accurately determine the PMI estimation in forensic cases by forensic entomologists.

ACKNOWLEDGEMENTS

I would like to thank my supervisors Prof Maureen Coetzee and Miss Allison Gilbert for all their support and guidance throughout the duration of my research. I would also like to thank Dr Guinevere Gordon who was one of my supervisors until illness prevented her from seeing this through to the end. I wish her the very best and a full recovery. Her encouragement with this rewrite has been hugely beneficial especially when I felt very lost.

I would like to acknowledge the National Research Foundation (NRF) for supporting this research through their Scarce Skills Fund and via the DST/NRF Research Chair Initiative grant to Prof Coetzee.

I would like to extend my gratitude to all the staff at the Forensic Pathology Service for all their assistance during autopsies and my insect sampling as well as during crime scene attendance. I would also like to thank the staff and students at the Vector Control Reference Laboratory for their help during my night-time laboratory work, especially Dr Shüné Oliver.

I would like to thank my colleagues at the Forensic Pathology Service, both past and present that encouraged me and aided me throughout this research, especially Mrs Carrie Bach who gave me many tips during the early stages of my data collection.

I would like to thank my family and friends for their encouragement, love and support through all the ups and downs of this research. A special thanks to my girlfriend for always pushing me to strive for the best and for believing in me.

Finally, I would like to thank David Smith for editing my English and grammar, as well as my examiners for their services in reviewing this dissertation, and their helpful comments in the previous iteration.

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CHAPTER ONE

Introduction, Literature Review, and Aims and Objectives

1.1 Introduction

Forensic entomology is defined as the use of insects and other arthropods in medico-legal investigations (Hall and Huntington, 2010). The primary function of the forensic entomologist in homicide investigations is the determination of the post-mortem interval (PMI) (Villet, 2011) or the estimated time of death. This PMI estimation is calculated using entomological and environmental data based on insect species, mainly Diptera and Coleoptera, associated with the human body as well as the biology and the ecology of those insects. Forensic entomologists can also provide additional insight into the circumstances surrounding the death and post-mortem treatment of the human body, for example if the body has been moved after death (Catts, 1992).

Different forensically important insect taxa are drawn to the dead and decomposing human body depending on their biological requirements, and the inherent benefits the body provides for those insects. These benefits may be directly obtained from the body itself or indirectly as a result of other insect taxa. Smith (1986) identified four ecological communities that are associated with a dead body based on the biology of those communities: necrophagous species feeding on carrion and human remains; predators and parasites of the necrophagous species; omnivorous species feeding on both the decomposing body and its colonisers; and lastly species that use the body merely as an extension of their environment.

Insects which are attracted to a decomposing body follow a somewhat predictable succession depending on the stage of decomposition of the body (Payne, 1965; Gennard, 2007). Dipteran taxa of the families Calliphoridae, Sarcophagidae, Phoridae and Muscidae have been found to be strongly associated with the stage of decomposition of both human and animal remains (Braack, 1981; Byrd and Castner, 2010; Villet, 2011; Gilbert, 2014). Some insects, however, are attracted to the corpse as a result of the expulsion of bodily waste (urine and faeces) and are therefore not always indicative of the time since death as they may have been present for some time before death occurred (Benecke and Lessig, 2001).

Environmental variables that affect dead bodies and that vary over time, such as temperature, humidity, and random events such as scavenging, fire and rain, must be taken into account before the collection of insects on the cadavers (Catts, 1992). These environmental disturbances may affect the insect taxa that colonise the remains, through increased mortality, or even prevent their colonisation completely. Evidence of such inhibitory factors may, however only be noted at the death scene unless detailed information is present at the autopsy.

The accurate estimation of the PMI is vitally important for criminal investigations. However, if forensic entomologists are not available at the death scene, the accuracy of the PMI estimation may be affected as important specimens may not be collected (Huntington *et al.*, 2007). The collection of specimens forms only part of the important data that are essential to estimating the PMI (Byrd *et al.*, 2010). Environmental factors, such as temperature, the amount of sunlight the body is exposed to, humidity, rainfall, and other micro-climatic variables, all affect the insects present in that environment, either promoting or inhibiting their colonisation of the remains (Smith, 1986; Castro *et al.*, 2011).

Removal of the body from the death scene may potentially cause secondary disturbances that could affect the insects that have colonised the remains. During the normal removal of the body, it is placed in a body bag, transported to the medico-legal mortuary and refrigerated before autopsy. These varying conditions may affect the insect colonisers. Refrigeration before autopsy is especially important to consider as it may affect the PMI estimation through changes in the developmental rates of the insects present on the body. Insects which are intended to be used for evidence in death investigations can be readily collected during autopsy when there is sufficient time and expertise available (Huntington *et al.*, 2007).

Therefore the temperature and duration of time (both of which are used in PMI estimation calculations) spent in a mortuary refrigerator is of critical importance (Huntington *et al.*, 2007; Byrd *et al.*, 2010). The duration and temperature of refrigeration may affect the PMI estimation as a result of a number of factors which need to be carefully recorded and considered when calculating the PMI (Johl and Anderson, 1996; Myskowiak and Doums, 2002; Huntington *et al.*, 2007). These factors include temperature deviations, insect activity, and the subsequent after-effects of the cooling of those insects. There is hence a need to understand the quality of the cold storage and cooling to allow for the aforementioned factors.

Forensic entomology therefore entails the estimation of the time of death using the PMI or time since colonisation. This estimation is, however, affected by numerous environmental variables at the death scene which need to be carefully considered to ensure that accurate

estimations can be determined by the forensic entomologist. Dipteran and coleopteran species are the most common insects associated with dead and decaying human remains (Smith, 1986; Villet, 2011). These are additionally the most important orders in PMI estimation from collections from the deceased (Villet, 2011). Forensic entomology in South Africa is a field which is growing but it's broader real-life application has been minimal within the South African medico-legal framework (Williams and Villet, 2006).

Therefore, the present study aims to evaluate the factors affecting colonisation and PMI estimation from samples taken at the post-mortem autopsy. This dissertation will describe the bodies from which the samples were collected and the death scene data which were available, and subsequently make recommendations regarding the importance of accurate death scene data to aid in estimating the PMI.

1.2 Literature Review

1.2.1 Decomposition

After death, the body begins the process of decomposition, which is the gradual process of autolysis and putrefaction leading to the dissolution of the soft tissue of the body (Saukko and Knight, 2004; Shkrum and Ramsay, 2007). There are four main types of decomposers involved in the process including: bacteria, enzymes, fungi, and scavengers (such as insect and vertebrates scavengers) (Saukko and Knight, 2004; Goff, 2009). Enzymes (internal), bacteria (both internal and external) and fungi (external) are responsible for the autolysis of cells and tissue (Saukko and Knight, 2004; Shkrum and Ramsay, 2007). Putrefaction is the liquid and gaseous transformation of the tissues by the bacteria and fungi (Shkrum and Ramsay, 2007). Insects and vertebrate scavengers contribute to the rapid removal of flesh (Shkrum and Ramsay, 2007; Goff, 2009). These decomposers drive the process of decomposition and the changes observed throughout each stage in decomposition.

1.2.2 Stages of Decomposition

After death, the body undergoes a number of post-mortem changes. These changes are driven by environmental variables as well as the time elapsed since death (Goff, 2009). They include

both chemical and physical changes that ultimately alter the appearance of the body. Very often these post-mortem changes are divided into clearly delineated stages in an attempt to define them based on observable features (Saukko and Knight, 2004; Goff, 2009; Payne-James *et al.*, 2011). However, as described by Goff (2009) these stages are artificial and exist more as a continuum. It is for the sake of simplicity that the stages are still used.

Many authors use five transitional stages in the process of decomposition, such as Anderson and Van Laerhoven (1996) who identified the stages as: fresh, bloated, active decay, advanced decay and dry remains. Similarly, Goff (2009) identified the same number of stages with some differences: fresh, bloated, decay, post-decay and skeletal. Payne (1965) described six stages of decomposition (fresh, bloated, active decay, advanced decay, dry stage and skeletal/remains) where some of his stages were combined by other authors, such as in Anderson and Van Laerhoven (1996), combining the dry and skeletal stages, and (Goff, 2009) combining the active decay and advanced decay. Regardless of the number of stages, there are still observable changes to the body that allow different methods and definitions to be used to describe the process. For the purposes of this review, a combination of the definitions will be used.

Fresh Stage

The fresh stage is typically described as starting from the moment of death until bloating (Goff, 2009). However, the fresh stage should include only the minor changes while the body is fresh and therefore this review will split the typical definition of the fresh stage into two: the fresh stage and the early or pre-bloat stage (to be defined later). Shortly after death, the initial physical and chemical changes begin to occur including rigor mortis, livor mortis and algor mortis. Rigor mortis is a chemical change that results in a noticeable physical condition of the body. Rigor mortis is defined as the binding of the actin and myosin filaments of the muscle because of reduced cellular adenosine triphosphate (ATP) production that causes muscular stiffening (Saukko and Knight, 2004; Shkrum and Ramsay, 2007; Goff, 2009; Payne-James *et al.*, 2011). Rigor mortis is important as it may aid in the estimation of time since death, however the onset and duration of rigor mortis is affected by a number of variables, including temperature, which causes some degree of error (Saukko and Knight, 2004; Payne-James *et al.*, 2011). Livor mortis is defined as the post-mortem gravitational settling of blood within the superficial capillaries, owing to the cessation of circulation, resulting in a pink to bluish discolouration (Shkrum and Ramsay, 2007; Payne-James *et al.*, 2011). The distribution of lividity is important as it provides information regarding the position of the body and the location of pressure points (Shkrum and Ramsay, 2007; Payne-

James *et al.*, 2011). Algor mortis is one of the other early physical changes that occur shortly after death, defined as the cooling of the body as a result of the inability to produce metabolic heat (Shkrum and Ramsay, 2007; Goff, 2009). Algor mortis has previously been used to estimate the time of death as a result of the cooling of the body, however the number of variables which affect the cooling of a body confound this estimate and as a result increase the margin of error (Saukko and Knight, 2004; Payne-James *et al.*, 2011). Insect attraction to fresh bodies can occur within minutes of death, with blowflies (such as *L. sericata*, *Ch. marginalis* and *Ca. vicina*) laying eggs around the orifices of the head (Sutherland *et al.*, 2013). Following the initial changes, the body begins to truly decompose (putrefy) and enters the early stage of decomposition where discolouration and odours increase.

Early Stage – Pre-Bloat Stage

The early stage is usually described as part of the fresh stage as mentioned previously. There are however noticeable differences between fresh bodies and those that have begun putrefaction without signs of bloating. The most obvious change is the blue-green discolouration of the body as a result of the haemolysis and the production of hydrogen sulphide gas from anaerobic bacteria (Shkrum and Ramsay, 2007), which reacts with the haemoglobin to form sulfhaemoglobin (Saukko and Knight, 2004). This discolouration is most notable in the lower abdomen but eventually spreads throughout the rest of the body (Shkrum and Ramsay, 2007). The blood vessels may also become discoloured by the same bacterial activity that results in a discolouration of the superficial blood vessels forming branch-like patterns on the skin known as marbling (Shkrum and Ramsay, 2007; Goff, 2009; Payne-James *et al.*, 2011). Other changes which may be associated include blistering (gas or fluid filled blisters as a result of the loss of skin integrity) and tache noir (drying of the cornea from the eyes being left open resulting in a red to black discoloration of the eye) (Shkrum and Ramsay, 2007; Goff, 2009). As the production of gases increase, a noticeable distension of the abdomen begins, forming the start of the bloat stage. Typically, flies of the families Calliphoridae (blowflies) and Sacrophagidae (fleshflies) are attracted to the body during this stage where they begin to oviposit (lay eggs) around the natural orifices of the head (Payne, 1965; Byrd *et al.*, 2010). Other families such as Muscidae (Muscid or house flies) may also colonise early because of their ubiquitous nature (Zumpt, 1965; Smith, 1986).

Bloat Stage

A number of gases are produced during decomposition which permeate into the soft tissue and organs causing swelling and distension of various parts of the body (Shkrum and Ramsay,

2007; Payne-James *et al.*, 2011). Swelling occurs mainly in the genitalia, abdomen, breasts and face (protrusion of the tongue and bulging of the eyes) (Shkrum and Ramsay, 2007; Payne-James *et al.*, 2011). Payne (1965) noted that the scrotum swelled first in piglets during the bloat stage. As a result of the increased pressure caused by the bloating of the abdomen, fluids are often purged through the nose and mouth and on occasion, urine and faeces through the genitals and anus (Saukko and Knight, 2004; Payne-James *et al.*, 2011). The bloat stage ceases when no further expansion can occur and the gases are expelled and the body deflates (Shkrum and Ramsay, 2007). This is as a result of the degradation of tissue and insect feeding activity which allows the gases to escape (Payne, 1965). Calliphoridae and Sarcophagidae maggot numbers increase greatly during this stage because of increasing colonisation by those families on the body (Payne, 1965).

Active decay

The active decay stage occurs through the combined effects of bacterial activity and maggot feeding (Goff, 2009). Gases have completely escaped from the body at this stage. The colour of the skin may gradually change from dark green to blackish-brown (Saukko and Knight, 2004). At this stage, insect activity (predominantly maggot activity on the body and fly activity around the body) is at its greatest, often with the formation of maggot masses (Payne, 1965; Saukko and Knight, 2004; Goff, 2009). The maggots secrete a proteolytic enzyme that speeds up the destruction of the tissue, greatly increasing the decomposition rate (Saukko and Knight, 2004). The internal organs (which often decompose slower than the outer surface of the body (Saukko and Knight, 2004)) progressively putrefy with some organs and tissue decomposing faster than others (Shkrum and Ramsay, 2007). Maggot activity will gradually move from the natural orifices to the thoracic and abdominal area (Payne, 1965). This leads to considerable loss of tissue and organs as the majority of the tissue is removed through maggot feeding activity (Goff, 2009). At the end of this stage the majority of the wet tissue has been consumed or putrefied and maggots migrate away from the body to pupate (Payne, 1965; Goff, 2009), leaving predominantly dry flesh, hair and bones.

Dry Stage

The dry stage is characterised by the presence of only dry skin, cartilage and bone (Payne, 1965; Goff, 2009). During this stage Coleopteran beetles are dominant and responsible for the removal of the remaining dried flesh reducing the body to skeletal remains (Payne, 1965; Goff, 2009). This dominance is a result of the decreased Dipteran activity as the dried flesh becomes unsuitable for their consumption, but facilitates the increasing numbers of

Coleopterans that thrive on the drier flesh (Smith, 1986; Byard *et al.*, 2010; Byrd and Castner, 2010).

Skeletal or Remains Stage

The skeletal stage is the final stage where only bone and hair remains (Goff, 2009). Payne (1965) found that it was impossible to distinguish where this stage began and the dry stage ended. The overall time to reach skeletonisation depends greatly on the environment and thus the season (Saukko and Knight, 2004). In typical decomposition of human remains, this is where the process ends as the bones may take years to degrade through desiccation.

1.2.3 Variations in Decomposition

There are a number of variations in the process of normal decomposition because of the environmental conditions acting on the body. These variations typically comprise processes that result in the preservation of the body, such as mummification and formation of adipocere.

Mummification

Mummification occurs in environments that are dry where bacterial activity is inhibited and the body desiccates instead of putrefying which results in a dry leathery appearance of the body (Goff, 2009; Payne-James *et al.*, 2011). The process of mummification may take a considerable length of time and the body may remain in this state for many years or until consumed by scavengers (especially necrophagous beetles which thrive on the dried flesh) (Saukko and Knight, 2004; Payne-James *et al.*, 2011).

Adipocere

Adipocere occurs when the body fat is hydrolysed to a waxy or greasy product initially (Shkrum and Ramsay, 2007; Payne-James *et al.*, 2011), but after many months it becomes brittle and chalky (Saukko and Knight, 2004). Typically adipocere is grey to white in colour and is irregular across the body (because of the distribution of body fat), but may affect the whole body (Saukko and Knight, 2004). Adipocere usually occurs in moist environments (including buried bodies in water logged or high clay soils) and in individuals that are obese or female (as a result of their naturally higher fat percentage compared to males) (Saukko and Knight, 2004; Shkrum and Ramsay, 2007).

1.2.4 Pattern of decomposition

As a body decomposes there is a typical pattern of decomposition observed. The head is the first region to reach a more advanced stage (Cross and Simmons, 2010; Gruenthal *et al.*, 2012), probably as a direct result of adult flies oviposition preferences in and around the natural orifices of the head, resulting in the flesh of the head being rapidly consumed by the maggots (Payne, 1965; Goff, 2009). Decomposition then moves down the neck towards the torso (Gruenthal *et al.*, 2012), following the movement of the maggots down towards the abdomen (Payne, 1965). The last area where flesh remains for some period is the limbs (Gruenthal *et al.*, 2012).

Charring of human remains affects this typical pattern causing the torso to decompose first followed by the head, then the neck and finally the limbs (Gruenthal *et al.*, 2012). Any factor that alters the condition of the body may alter the rate and pattern of decomposition and subsequently the rate of succession and/or insect activity and arrival time.

1.2.5 Factors affecting decomposition

The decomposition of a body can be affected by a number of factors through both altering the decomposition itself and hindering the arrival of insects (Goff, 2009). These include physical, chemical and climatic factors. Physical barriers (e.g. bodies which are located in an enclosed or indoor environment such as a house or motor vehicle, bodies immersed in water, buried bodies, and covered bodies) may not affect all aspects of the decomposition but they will prevent access to the body by decomposers such as insects and hence slow the rate of decomposition (Goff, 2009). Chemical barriers such as embalming (affecting decomposition and colonisation) and the use of insecticides (affecting colonisation) may have considerable effects for quite a period of time (Goff, 2009). Seasonal and environmental factors, such as temperature, rainfall, and wind, can have a number of effects on both the decomposition of the body and the colonisation by insects (Goff, 2009).

Insects

Insects are important in the process of decomposition (Saukko and Knight, 2004; Goff, 2009; Simmons *et al.*, 2010a). In a study by Simmons *et al.* (2010a) it was found that insect

presence had the greatest effect on the rate of decomposition. The overall process of decomposition and the rate of decay are dependent on the presence of insects in conjunction with temperature (Payne, 1965; Sutherland *et al.*, 2013). Insect are, however, sensitive to a number of other factors which affect their presence and ability to colonize the body including competition, disturbance of the body, sunlight and humidity (Smith, 1986; Byrd and Castner, 2010)

Temperature

Temperature has a considerable effect on decomposition. High temperatures can promote the increased enzymatic and bacterial activity and growth of the naturally occurring fauna of the body, rapidly increasing decomposition (Saukko and Knight, 2004). Cooler temperatures decrease this growth and activity and hence decrease the rate of decomposition (Saukko and Knight, 2004). High temperatures also increase insect activity which results in more rapid tissue depletion (Sutherland *et al.*, 2013). The inverse of this is also true: as temperatures decrease insect activity decreases until development ceases (Higley and Haskell, 2010). Both insect activity and development are affected by temperature (Castner, 2010). As a result, temperature drives decomposition through both enzymatic and bacterial action, and insect activity. Temperature therefore has an effect on both the duration and onset of all the stages of decomposition. (Saukko and Knight, 2004; Goff, 2009). The specific effects of temperature on insects and the implications for estimating the PMI are discussed later in this review.

Location of the body

Decomposition of a body in an indoor environment is often slower than that of outdoor environments (Simmons *et al.*, 2010a; Anderson, 2011). This slower decomposition is a combined effect of the inhibition of insect colonisation which results in decomposition of indoor bodies being greatly extended (Anderson, 2011). Immersed bodies typically decompose slower than those on land due to a number of reasons, including the lower average temperature of water, exclusion of terrestrial insects, and on occasion the formation of adipocere (Saukko and Knight, 2004; Payne-James *et al.*, 2011). Buried bodies decompose slower than outdoor, indoor and immersed bodies (Saukko and Knight, 2004; Payne-James *et al.*, 2011). In some cases decomposition may be delayed for a considerable length of time (Saukko and Knight, 2004). As described by Saukko and Knight (2004) there are a number of factors that affect the decomposition of buried bodies including: lower average temperatures than other environments, exclusion of scavengers and insects, and lack of oxygen. It is the exclusion of insects that has been found to be the predominant limiting factor in

decomposition (Simmons *et al.*, 2010b). In cases where insect colonisation occurs before burial there is a faster rate of decomposition than complete exclusion (Simmons *et al.*, 2010b). The soil characteristics may have further effects such as clay-type soils with high moisture content promoting the production of adipocere or dry sandy soils which may aid in mummification (Saukko and Knight, 2004). Simmons *et al.* (2010a) found that the decay rates of indoor, buried, and submerged bodies were in fact not significantly different from each other when measured using accumulated degree days (ADD) and they concluded that the presence of insects was the main factor driving the rate of decomposition.

In a study conducted by Fitzgerald and Oxenham (2009) on the difference between pigs decomposed in full sun compared to shade, they found that there were significant differences between the decomposition in sunlight and shade. The scope of this study did not include insects but differences were noted between the two pigs concerning the insect activity where greater maggot mass formation and maggot numbers were noted on the 'full sun' pig.

Location, therefore, is an important feature but the presence of insects appears to be the most important factor with regards to how the location affects their ability to colonise the body (Simmons *et al.*, 2010b).

Covered or clothed bodies

Covering a body, whether with clothing or wrapped in plastic, results in a decrease in loss of moisture and therefore extends the period of decomposition compared to bodies that are exposed or not covered in any way (Kelly *et al.*, 2009). Matuszewski *et al.* (2014) however, found that clothing had no significant effect on the onset and duration of the different stages of decomposition except in the later stages. This agrees with the findings of Kelly *et al.* (2009) as clothing (or wrapping/covering) appears to provide some level of protection for the insects and may favour putrefaction (Matuszewski *et al.*, 2014).

Burning

Burning to the point of charring initially creates an artificial appearance of a more advanced stage of decomposition, however, the overall rate of decomposition in burned bodies is no different to that of unburned bodies (Gruenthal *et al.*, 2012). Gruenthal *et al.* (2012) found that even though the rate was not different there was a significant effect on the pattern of decomposition because of burning. Body regions where charring was present were found to decompose faster, while those with lighter levels of charring decomposed slower.

Body Size and Mass

Smaller bodies decompose faster than larger bodies (Simmons *et al.*, 2010a; Sutherland *et al.*, 2013). Simmons *et al.* (2010a) suggested that this was only the case when insects have access to a body and that the exclusion of insects would result in no difference. The authors suggested that the smaller body size (hence less tissue present) would allow insects to remove flesh more rapidly.

But the overall mass of the body has a more complex effect on decomposition. Bloating starts earlier and lasts longer in larger and heavier bodies (Matuszewski *et al.*, 2014). Larger carcasses also have a longer active decay stage, which is predominantly driven by insect activity (Matuszewski *et al.*, 2014). Larger bodies initially decompose faster, possibly as a result of their advanced age and more developed intestinal fauna, but thereafter the smaller and lighter bodies decompose faster (Matuszewski *et al.*, 2014).

Scavenging

Scavenging by larger mammals such as canines can have considerable effects on decomposition through the removal of flesh, clothing and identifying features in addition to the removal of maggots which may result in the bypassing of entire stages of decomposition (Shkrum and Ramsay, 2007; Anderson, 2010).

1.2.6 Forensic Entomology

As stated above, insects have a very close association with a dead body, often arriving shortly after death (Anderson, 2011; Sutherland *et al.*, 2013). When insects are present they usually remain associated with the body throughout decomposition, until the body has been reduced to dry bones (Payne, 1965; Smith, 1986). It is the close association of certain insect families to the different stages of decomposition of human remains that forms the basis of forensic entomology (Payne, 1965; Smith, 1986).

Forensic entomology, often referred to as medico-legal entomology, is defined as the study of insects and other arthropods associated with human remains for the purpose of aiding in the investigation of death (Smith, 1986; Hall and Huntington, 2010). The primary role of the forensic entomologist is in the determination of the time elapsed since death (Smith, 1986; Catts, 1992; Amendt *et al.*, 2004; Hall and Huntington, 2010). Secondary roles include

providing information that may allude to the circumstances surrounding the death, from the time of death to the time of discovery. These roles include providing information regarding movement of the deceased (Catts, 1992), and the presence of toxins in the deceased (entomotoxicology) (Gosselin *et al.*, 2011).

Insects, especially Diptera (flies), are attracted to a body soon after death (Payne, 1965; Smith, 1986; Smith, 1989; Amendt *et al.*, 2004). The Diptera are one of the most adaptive insect orders and as a result occur in almost every environment and ecological niche (Smith, 1989). The fly larvae are predominantly protein feeders and as such many species have considerable medical importance where larvae may be present in either dead or living tissue (termed myiasis when they feed on living human tissue) (Zumpt, 1965). Dipteran larvae which cause myiasis may be either obligate parasites (complete development in or on their host) or facultative parasites (free-living and usually develop on decomposing organic matter but may complete part of their development on the host) (Zumpt, 1965). Myiasis may be the initial association of some larvae with the body before death, but this is the exception rather than the rule in the case of insect associations with the dead. The dipteran families commonly associated with opportunistic/facultative myiasis are also the most important decomposers, these being the Calliphoridae and Sarcophagidae families (Smith, 1989), which are commonly attracted to decomposing bodies.

Insect attraction to a body is largely as a food source or habitat (Gennard, 2007). As such the body forms an important part of the insect's environment and hence its ecology (the relationship between an organism and its environment). The attractiveness of the body depends on the insect species and the resources available over time (Archer and Elgar, 2003).

1.2.7 Insect Association to bodies

Insects and other arthropods that associate with a carcass are responsible for performing a number of roles in relation to both the carcass itself and the other arthropods present. These roles were initially described by Payne (1965) and later described further by Smith (1986) and Goff (2009). These roles include:

1. Necrophagous species –arthropods feed directly on the decomposing tissue of the body and are the most important group in estimating the time of death.

2. Predators and parasites – those that are attracted to the body by the presence of the other arthropods to feed on or parasitize them.
3. Omnivorous species – arthropods that feed both on the body and the other arthropods present on the body. Includes generalist feeders as well as those with specific life strategies.
4. Adventive species – arthropods that use the body for shelter or as an extension of their habitat.
5. Accidentals – Arthropods that are present by chance, e.g. by falling on the body or landing on the body by chance.

Each of these roles affects which insect families will arrive at the body based on a number of factors that attract the insects to colonise the body.

1.2.8 Insect attraction and colonisation

Necrophagous insects are attracted to the body within minutes of the body becoming exposed (Sutherland *et al.*, 2013). These insects colonise the natural orifices, which emit the odours of attraction (Cross and Simmons, 2010; Sutherland *et al.*, 2013). Sites of trauma have also been suggested to be sites of colonisation or initial oviposition by Diptera (Castner, 2010). The natural orifices will always be selected over any other site (Cross and Simmons, 2010). Second to this, the natural skin creases are preferred over sites of trauma (Cross and Simmons, 2010). Charabidze *et al.* (2015) found that wound sites were never selected for oviposition and only orifices or surface contact sites were chosen. This attraction to the natural orifices is believed to be the result of volatile chemicals of decay from the gut being released through these areas (Cross and Simmons, 2010).

Stage of decomposition and odour

In the early stages of decomposition there is little or no noticeable odour (Payne, 1965; LéBlanc and Logan, 2010). Regardless of the lack of detectable odour by researchers, insects are still attracted to the body soon after death. Insects have highly specialised chemoreceptors with which they are able to detect semiochemicals emitted from the body even when no odour is detected by humans (LéBlanc and Logan, 2010). Bacteria within the gut begin to break down the soft tissue leading to putrefaction of the body. Hydrogen sulphide, methane and sulphur dioxide are produced as a result. These gases lead to discoloration of the abdomen

and blood vessels at this stage but produce little odour (LéBlanc and Logan, 2010). As the bacteria continue to produce gases, the body begins to bloat, predominantly in the abdomen, and purges fluids from the mouth, nose and rectum. An obvious odour is produced at this stage (LéBlanc and Logan, 2010), which smells strongly of ammonia (Goff, 2009). During the bloat stage Dipteran numbers start increasing (Payne, 1965) reaching a peak by the end of the stage (Kreitlow, 2010). As the gases produced by the bacteria begin to escape, a more active stage of decomposition is reached. Strong odours are produced by sulphur compounds and inorganic gases produced in the gut (LéBlanc and Logan, 2010). This stage has a strong putrid odour that is the strongest odour of all the stages. Dipteran larvae form maggot masses during this stage and actively increase their rate of feeding, removing large quantities of soft tissue. Large numbers of predators are attracted by the maggot masses (Payne, 1965; Kreitlow, 2010). Once most of the soft tissue has been consumed the body enters an advanced decay stage where the odours are less marked but still noticeable (LéBlanc and Logan, 2010). Dipteran larvae numbers are greatly reduced as most have migrated away from the body to pupate (Kreitlow, 2010). By this time only dry flesh and bones typically remain. A slight odour of dried skin is noticeable (Payne, 1965; LéBlanc and Logan, 2010). At this stage, beetles which feed on the dried flesh become dominant (Kreitlow, 2010). Once the beetles have removed the last of the dried flesh the body enters the skeletal stage. Low odours are produced at this stage and no more successive species occur (Payne, 1965).

Each of the aforementioned stages are very important in the production of chemical cues to attract the relevant successive insect waves to the decaying body. LéBlanc (2008) conducted a study measuring the amount of semiochemicals produced at each stage of decomposition. It was found that odour occurs in direct relation to the concentration of semiochemicals. These chemicals provide information regarding the location of the body as well as the suitability (resources available) for each insect species and its offspring (LéBlanc and Logan, 2010). These chemical cues attract certain dipteran and coleopteran species at certain times and repel others at other times, supporting the significant relationship of species occurrence with the stage of decomposition (Smith, 1986; Archer and Elgar, 2003; Gennard, 2007). The adults of some dipteran and coleopteran species will visit the body even when they will not colonise it (Archer and Elgar, 2003; Michaud and Moreau, 2009). This may be as a result of residual semiochemicals still present or for the purposes of finding mates (Archer and Elgar, 2003).

1.2.9 Succession

The attraction to specific odours forms the basis of niche formation or insect succession (Smith, 1986; Byrd *et al.*, 2010; LéBlanc and Logan, 2010). Succession is based on the concept of ecological succession which is the orderly and predictable changes in species composition of an ecological community over time (Kreitlow, 2010). These changes in the ecological community are caused by changes in environmental factors (geographic region, season, habitat and weather conditions), food sources, intra and interspecies competition, and other random events such as fire (Gruenthal *et al.*, 2012), which cause succession to progress or alter the succession in some way (Anderson, 2010; Kreitlow, 2010). The human body, as it decomposes, supports a large number of insect species, predominantly as a food source, and therefore forms a complex and dynamic ecosystem (Anderson, 2010).

There are a number of factors that can affect the successive colonisation by insects including: the location of the body (geographical, indoor vs outdoor), covering of the body, time of day, season (including temperature), disturbances and predators or scavengers. These may affect the way in which the chemical cues permeate or may provide physical or environmental barriers preventing colonisation by insects.

Geographical location

Insect colonisation in an area is affected by a number of factors including the habitat, vegetation, soil type and meteorological conditions (Anderson, 2010). All of these factors form the biogeographical zone and affect not only the insect colonisation but also the way in which the body will decompose. These factors are different for different regions and as such cannot be applied in a universal manner (Anderson, 2010). Insect colonisation therefore needs to be studied for each specific geographic location to ensure that all the aforementioned factors are considered for that location.

Indoor vs Outdoor

Outdoor bodies are colonised faster and decompose faster than indoor bodies (Anderson, 2011). Indoor environments inhibit the penetration of odours to the outside which delays the arrival of the insects and hence cause the body to be colonised later than outdoor environments (Byrd *et al.*, 2010; Anderson, 2011; Sutherland *et al.*, 2013). Pohjoismäki *et al.* (2010) found that in many cases of decomposition from indoor environments sent for autopsy, there were no signs of insect activity because of the lack of access by the insects to the body.

As such, outdoor cases are often noted to have more species present than indoor cases (Schroeder *et al.*, 2003; Anderson, 2011). In cases where the odours are able to penetrate outdoors but the insects are unable to gain access, their presence may be noted in the surrounding area (Byrd *et al.*, 2010). It has been observed that *Lucilia sericata* is one species that has the tendency to enter indoor environments (Pohjoismäki *et al.*, 2010). Goff (1993) similarly states that the presence of insects on a body are only reflective of that location, habitat or because of the stage of decomposition. This may be a specific life strategy to reduce competition through different habitat selection (LéBlanc and Logan, 2010) or the greater ability of those species to detect semiochemicals (Anderson, 2010).

Sun vs Shade

Archer and Elgar (2003) studied the effect of shade on insect colonisation. They found that pigs in semi-shaded areas had a greater number of insects present and higher species diversity. They did however note that the rate of succession was considerably slower in semi-shaded pigs. Castro *et al.* (2011) also found that shaded pigs had a greater number of species. They concluded that the slower rate of decay resulted in more time for colonisation in the later stages of decomposition. Pigs in full sun decompose faster than those in shade, with high numbers of Calliphoridae present in the late stages of decomposition (Castro *et al.*, 2011). Fitzgerald and Oxenham (2009), however, found that there was no difference in the overall rate of decomposition even though insect colonisation does appear different. Insect colonisation may vary under these conditions but it is clear that the microclimatic variables need to be considered, to clearly highlight any differences (or lack thereof).

Clothing or covering

Kelly *et al.* (2009) found that wrapping or covering a body did not affect the colonisation except in winter. Wrapping appeared to affect the drying of the body by maintaining the moisture of the body. Wrapping the body facilitated the movement of maggots allowing them to distribute to other areas of the body more easily. This is probably because of the protected microclimate created by the wrapping of the body.

Time of day and light

Colonisation by blowflies is typically during daylight (diurnal) with most species becoming inactive at night (Anderson, 2010). Darkness has been noted to inhibit the egg laying behaviour in most species, however some species will actively seek dark areas to oviposit (Anderson, 2010). Payne (1965) found that insect activity gradually decreased at night in all

stages of decomposition, with some Coleopterans being the only exception, highlighting the predominant diurnal activity of most colonisers.

Seasonality

Calliphoridae and Sarcophagidae are the dominant fly families in all seasons and are usually the first to colonise bodies (Bharti and Singh, 2003). Warmer seasons (summer and spring) have higher insect activity because of the higher temperatures and bodies found during these seasons often have a greater number of colonising dipteran species than bodies found in colder seasons (Schroeder *et al.*, 2003). Many dipteran species also display specific seasonal activity, where they increase in numbers in certain seasons but become relatively inactive in others (Archer and Elgar, 2003; Schroeder *et al.*, 2003; Byrd *et al.*, 2010). Bharti and Singh (2003) in a study conducted in Punjab, India found that spring had the highest species diversity. They concluded that this is a result of the moderate temperatures allowing both late winter and early summer species to be active. This result should be further tested in different areas to determine if the trend seen is a general one.

Disturbances

De Jong and Hoback (2006) studied the effects of disturbances on colonisation and found no significant impact on the taxa present. This has been found not to be the case by other authors (Adlam and Simmons, 2007; Cross and Simmons, 2010), however these authors moved and weighed the pigs to estimate weight loss over time and not disturbance. Scavenging by larger mammals may also have considerable effects on decomposition through the removal of maggots (Shkrum and Ramsay, 2007; Anderson, 2010). Therefore, the effect of disturbance requires more research to be better understood.

Predators or scavengers

Predators (wasps, beetles, ants, birds and mammals) may be responsible for removing insect evidence and may alter the succession and presence of certain species as a result. Scavenging by insects and animals can also have considerable effect on the decomposition of the body through the removal of not only flesh but the insect colonisers which may result in entire stages of decomposition being bypassed or colonisation of some stages being missed completely (Anderson, 2010). Predation of other insects then may alter the overall sequence of succession (Gennard, 2007). Ants are opportunistic feeders which may be present at any stage of decomposition where they may feed on the adults and larvae of flies and beetles as well as the decomposing flesh (Smith, 1986; Campobasso *et al.*, 2009). Ants are therefore

both predators and scavengers (Smith, 1986). The overall effect of ants on the decomposition is dependent on their numbers and the geographic area (Goff, 1993; Anderson and Van Laerhoven, 1996).

All of the abovementioned factors are important as they can have an effect on the succession or colonisation of the insects associated with a body. It is well established that through the study of the assemblages of arthropods it is possible to estimate the time elapsed since death (Smith, 1986; Anderson, 2010). Succession can be an excellent method to estimate the time since death but a holistic approach is needed to completely account for factors affecting the timing and species composition (Anderson, 2010).

1.2.10 Estimating the time since death

Estimation of the time since death (often referred to as the PMI – post-mortem interval) depends greatly on the time of discovery of the corpse and collection of specimens (Goff, 2009). Estimations begin at the time when specimens are collected and preserved (Goff, 2009). These estimates are not precise and the precision decreases with an increase in the time since death (Goff, 2009), and are in fact not estimating the time since death but rather the time of colonisation (Villet, 2011). Therefore these estimates require an experienced entomologist (Saukko and Knight, 2004; Payne-James *et al.*, 2011), as the estimation is only possible with an accurate identification, understanding of the biology and the ecology of the insect species involved (Smith, 1989; Villet, 2011).

When a prolonged period of time has passed and the majority of the early post-mortem changes used by the pathologist are no longer applicable, the presence of insects may provide the most reliable estimation of PMI (Gennard, 2007; Anderson, 2010). For this reason, the estimation of the PMI is the primary role of the entomologist in death investigations (Smith, 1986; Goff, 1993; Anderson, 2011; Villet, 2011). The estimation of the PMI uses the succession of different species assemblages of Diptera and Coleoptera on the body (Rodriguez and Bass, 1983; Goff, 1993; Amendt *et al.*, 2007; Gennard, 2007), insect development and their lifecycle (Goff, 1993; Gennard, 2007).

The PMI is estimated using the maximum and minimum interval times between the potential time of colonisation and the time of discovery (and insect collection) (Goff, 1993; Goff, 2009). The maximum interval time is determined using the information from the succession

of insects on the body with respect to the factors affecting their colonisation (Goff, 1993). This provides a snapshot in time of the insects present, which can be compared to the species that should be present under similar conditions for that region and time of year (Gennard, 2007). Therefore, the succession model requires data of the local fauna from experimentation to provide an estimate (Rodriguez and Bass, 1983; Amendt *et al.*, 2007; Gennard, 2007). The minimum limit is determined by estimating the age of the developing larvae at the time of collection from the body (Goff, 1993). The larval age and development depends on the specific conditions of the location such as the geographic climate, season and weather. Post mortem interval estimations using this method are also known as the thermal summation method which uses the temperature dependent development of dipteran larvae (Hall and Huntington, 2010). Baseline studies for each region on the development of each species of maggot are important to reduce the margin of error in estimating the PMI (Goff, 1993). All the factors (time of oviposition, time of activity, weather, presence of toxins and scavengers) that affect the colonisation need to be considered when providing an estimate of the PMI (Goff, 1993; Villet *et al.*, 2010).

Even though the estimation of PMI from insects is regarded as one of the most reliable estimates, the delay in colonisation under certain environmental conditions increases the error in the estimation due to the interpretation of the results (Gennard, 2007; Cockle and Bell, 2015). Another compounding factor is that the estimation becomes even more inaccurate the more time passes (Amendt *et al.*, 2007). To reduce the subjectivity and inaccuracy of these estimates, a multidisciplinary multi-method approach has been suggested by a number of researchers (Ferreira and Cunha, 2013; Sutherland *et al.*, 2013; Cockle and Bell, 2015).

However, another factor which has received little attention is the effect of cold storage once bodies arrive at the morgue (Pohjoismäki *et al.*, 2010). This is often not a problem should insect collections occur at the death scene before autopsy, but often the collection of insect evidence is only done at the autopsy itself (Johl and Anderson, 1996; Huntington *et al.*, 2007), or even after the autopsy has been completed (Thevan *et al.*, 2010). If collections are only done at the time of autopsy, there are a number of factors that must be considered. Firstly, the ambient temperature at the death scene and the time that the body was removed is essential (Harvey *et al.*, 2016), and secondly, information regarding the storage of the body prior to the autopsy (time and temperature within cold storage) (Amendt *et al.*, 2007). The storage of the body (and associated insects) prior to autopsy and subsequent insect collection has serious implications for the forensic entomologist and the estimation of a PMI. Collecting insects from the death scene and obtaining scene temperatures would provide the most reliable PMI

estimation (Scala and Wallace, 2010). Failing to obtain such evidence, it may still be possible to estimate the PMI given accurately collected temperatures, time of removal from the scene, the period of time of the body in cold storage and time of collection of insects at the autopsy.

Much of this time would be spent in the mortuary refrigerator or cooler where the temperature and duration of cold storage are of critical importance when calculating the PMI (Huntington *et al.*, 2007; Byard *et al.*, 2008). The temperature and duration of cooling may affect the PMI estimation through temperature fluctuations and changing insect behaviour or rates of development (Johl and Anderson, 1996; Myskowiak and Doums, 2002; Huntington *et al.*, 2007; Thevan *et al.*, 2010; Villet *et al.*, 2010; Harvey *et al.*, 2016). Myskowiak and Doums (2002) found that a period of cooling could alter the overall rate of development (growth) of the maggot through the behavioural inactivity as a response to harsh conditions, known as diapause. This is essential to winter survival (in addition to other harsh conditions that may threaten the life of the insect) for many species (Myskowiak and Doums, 2002), and may occur during short term mortuary refrigeration. Refrigeration may have more of an effect on the development of the maggot than simply ceasing development, such as increasing mortality or altering development after refrigeration (Johl and Anderson, 1996). Without considering these effects, the estimation of the PMI may be considerably affected and inaccurate.

1.2.11 Accuracy of PMI Estimation

Estimations of the PMI using insect data depend on the time of arrival of adult flies and the oviposition of eggs on the body (Villet and Amendt, 2011). Once eggs have been laid on the body the developmental or physiological "clock" begins, which is known as the post mortem interval minimum (PMI_{min}) (Villet and Amendt, 2011). The physiological clock uses the temperature dependent development of forensically important taxa to assist in calculating a time since colonisation (Gennard, 2007; Villet and Amendt, 2011). The physiological clock is usually represented in degree-days or degree hours in forensic entomology (Gennard, 2007; Higley and Haskell, 2010; Villet and Amendt, 2011). The combination of time and temperature as a measure of development provides a very predictable method to estimate insect development given temperature. This development is dependent on the maximum and minimum developmental threshold temperatures. Below the minimum temperatures development stops, while above the maximum threshold the rate of development initially decreases before ceasing completely (Gennard, 2007; Higley and Haskell, 2010).

The development of any insect from the time of oviposition to the time of collection depends on the temperature of the death scene and the temperature of the body (Higley and Haskell, 2010; Villet and Amendt, 2011). Accurate estimations of the PMI require accurate measures of temperatures at the death scene and accurate developmental rates for those respective temperatures (Higley and Haskell, 2010). This is especially important when there has been a delay in the collection of insects from the body. If these insects are not stored in conditions that halt development completely, then it is essential to record the environmental conditions to which they are subjected to determine more accurate developmental estimates (Johl and Anderson, 1996; Higley and Haskell, 2010).

In a review by Sharma *et al.* (2015) the authors described a number of methods of PMI estimation and the associated limitations of each. The limitations identified included (among others) the effects of low temperatures in estimating the PMI from accumulated degree hours where periods of low temperatures could affect development but the precise time of this effect would be unknown and hence not accounted for. Other limitations included the variable time of oviposition, the preservation method when attempting to use larvae length as an age estimate and variable environmental or field conditions. When estimating the PMI it is important to consider the effects that may increase the margin of error in estimates (Higley and Haskell, 2010; Villet *et al.*, 2010; Villet and Amendt, 2011; Sharma *et al.*, 2015). It is therefore important to firstly record all possible conditions that insects are exposed to before collection and secondly to use a number of different PMI estimates (Higley and Haskell, 2010).

1.2.12 Forensic Entomology in South Africa

Numerous recent studies in South Africa into PMI estimations have been conducted by Prof Martin Villet (Richards and Villet, 2009; Villet *et al.*, 2010; Villet and Amendt, 2011), but there have been very few studies published on the insects frequenting human cadavers (Louw and van der Linder, 1993). The majority of the forensic entomological research in South Africa has been conducted on animal carcasses (Braack, 1981; Kelly *et al.*, 2009; Gilbert, 2014) or have utilised commercial fly traps to sample forensically important species (Williams, 2003; Richards *et al.*, 2009b). These studies have provided valuable information regarding the insect distributions throughout South Africa and insect colonisation of decaying animals in addition to the accuracy of PMI estimations. There is, however a need to study the

colonisation of human bodies to aid in the determination of PMI for cases under medico-legal investigation. Currently PMI estimations in South Africa are not routine in death investigations and estimates rely on the eyewitness information and broad estimates as given by the forensic pathologist, which become increasingly inaccurate as time since death progresses (Catts, 1992; Saukko and Knight, 2004). The role of the forensic entomologist in South Africa requires clarification and validation to grow into a more accepted forensic tool locally (Williams and Villet, 2006), and thereby grow the profession as a whole. Many of the South African mortuaries lack the facilities to accommodate full time forensic entomologists. This means that much of the growth in the field lies with the South African Police Service, who currently have a small unit attempting to serve all the cases for the country (personal communication).

Most (if not all) cases have no forensic entomology specimens collected until the time of autopsy (if at all). This means that valuable information is lost in the majority of cases where available insect evidence is not collected. Therefore, this research aims to analyse the cases received at the mortuary with insect colonisation present. Data, or lack thereof, obtained from the scene and the degree of error when estimating the PMI from insect samples taken only at the time of the autopsy, are presented.

1.3 Context and Outline of the dissertation

1.3.1 Context of the research

This study was conducted at the Johannesburg Forensic Pathology Service in Braamfontein. The role of the Forensic Pathology Service (FPS) is defined by the National Health Act No. 61 (2003) as:

- Performing a death scene investigation (this investigation is limited to the cause and circumstances surrounding the death and often no evidence other than that on the body is recorded, the police being responsible for collecting all other evidence)
- Obtaining information that may be relevant to the medico-legal investigation
- The collection and removal of the body
- Conducting the autopsy and collection of evidence from the body
- Requesting and conducting special investigations (including entomological analyses).

Forensic entomological analyses are not routine investigations and may only occur at the request of the investigating officer or forensic medical practitioner responsible for conducting the autopsy. It is to the discretion of the forensic medical practitioner, as the designated person, to request the attendance of other experts, including entomologists, to be present at the autopsy (National Health Act No. 61, 2003). This often means that at the time of autopsy the medical practitioner may not be aware of insect evidence present on the body until the autopsy commences and may therefore not request the presence of an entomologist. Some forensic medical practitioners may attempt to collect insect specimens at the time of autopsy but these are typically preserved in formalin.

The National Health Act No. 61 (2003) also states the requirement for the storage of the body within a temperature controlled refrigerator, however no guidelines for the set temperature are provided nor any other specifications.

1.3.2 Outline of the dissertation

This dissertation consists of two major chapters in the format of journal articles and are written as stand-alone articles. The first article (Chapter 2) provides a review of the decomposed bodies received at the Johannesburg FPS and the associated insect species present. The importance of this chapter is to provide information on the insects associated with bodies found in different locations, seasons, environments and stages of decomposition. The data presented will attempt to highlight the importance of scene information especially when insect evidence is needed for PMI estimation using succession as a model for PMI. Forensic entomology analyses are not routinely performed or requested and as such this article aims to highlight the important factors which need to be considered and recorded for use in investigating cases with insect succession.

The second article (Chapter 3) highlights the implication for PMI estimation based on the temperatures at the death scene, in refrigerators, and the autopsy room. A simulated approach of PMI estimation using Monte Carlo simulation was performed to demonstrate the uncertainty which is introduced when PMI estimations are only performed from insects sampled after the autopsy. This article considers the effect of PMI estimations considering the temperature variations which the body and associated insects experience after being removed from the death scene.

1.4 Aims and objectives

The aim of this study was to determine the factors affecting colonisation and PMI estimation from entomological collections performed at the post-mortem autopsy.

The specific objectives were as follows:

1. To describe the bodies observed in autopsy with respect to the stage of decomposition, associated insect species, scene data collected (including temperature) and time of year.
2. To determine the factors that predict the number of taxa present and the species which colonise the remains.
3. To determine if refrigeration before autopsy could affect the estimation of PMI of insect samples taken at autopsy.

CHAPTER TWO

A 16-month analysis of human remains and the factors affecting insect colonisation

2.1 Introduction

Early post-mortem changes, as the body progresses through decomposition, are important to the pathologist and anthropologist in estimating the post-mortem interval (PMI) (Fitzgerald and Oxenham, 2009; Payne-James *et al.*, 2011; Sutherland *et al.*, 2013). However these changes can be unreliable as they are affected by numerous internal (body size and weight, cause of death) and external factors (temperature, humidity, body coverings, scavengers and insects) (Amendt *et al.*, 2007; Payne-James *et al.*, 2011). When human remains are found more than a few days since death, many of the methods employed by the forensic pathologist during the autopsy to determine the PMI are no longer reliable or appropriate (Amendt *et al.*, 2004). In these cases, if insects are present on the body, they may provide the most reliable estimation of the PMI (Gennard, 2007; Hall and Huntington, 2010).

Many insect species have close associations with various stages of decay and are hence attracted to the body during these stages through faunal succession (Smith, 1986). This often results in numerous species and large numbers of insects being present on the body at the same time. Insect activity should therefore be carefully noted and the expertise of a forensic entomologist used in cases of advanced decomposition (Saukko and Knight, 2004). Much of the research in the field of forensic entomology has used animal models, since the use of human bodies for such experimentation poses severe ethical issues (Smith, 1986; Stokes *et al.*, 2013). The domestic pig (*Sus scrofa domestica*) is one of the most commonly utilised analogues as pig tissue has been found to be the most similar to human tissue (Stokes *et al.*, 2013). There have been a number of studies conducted in South Africa on forensic entomology using pigs (Kelly, 2006; Kelly *et al.*, 2009; Richards and Villet, 2009; Richards *et al.*, 2009b; Gilbert, 2014), however there is little recorded on the colonisation and insect diversity from a series of human medico-legal cases.

Medico-legal forensic entomology uses the colonisation of insects on human remains to estimate the time since colonisation to infer the post-mortem interval (PMI) (Villet *et al.*,

2010). The estimation of the PMI depends on a number of factors: insects may be physically inhibited from colonising by the location of the body, such as being found indoors (Pohjoismäki *et al.*, 2010; Simmons *et al.*, 2010a; Anderson, 2011), or through burial (Simmons *et al.*, 2010a). Insect colonisation may be prevented through physiological means, such as high or low temperatures preventing oviposition (Schroeder *et al.*, 2003; Danks, 2007; Villet *et al.*, 2010; Villet, 2011).

As a body decomposes it releases volatile chemicals in the form of gases as a result of bacterial activity. These act as chemical cues which attract certain species at certain times while repelling other species (LéBlanc and Logan, 2010). Each stage in the process of decomposition releases different chemical cues that result in a very specific pattern of colonisation or succession (Anderson, 2010; LéBlanc and Logan, 2010; Villet *et al.*, 2010). There are five generally accepted stages in decomposition: fresh, bloat, active decay, dry, and skeletal (Gennard, 2007; Goff, 2009). For the purposes of this study one additional stage was used, the early or pre-bloat stage. This stage is defined as the stage where the initial post-mortem changes of discoloration to the abdomen and marbling have occurred before any signs of bloating. This distinction was made as other studies have defined bodies as being decomposed only when discoloration was observed (Byard *et al.*, 2008; Keyes *et al.*, 2016). The odours produced at each stage begin as almost non-existent in the fresh stage, gradually increasing to the greatest point in the active decay stage, and then decreasing until nothing but the skeleton and hair remain (Payne, 1965; LéBlanc and Logan, 2010). It is this gradually increasing odour which attracts an increasing number of insects until the active decay stage where maggots form masses and remove the majority of the flesh (Goff, 2009). Thereafter the number of insects (especially Diptera) decline until the resources are completely consumed (Goff, 2009; Anderson, 2010).

This process of succession is affected by the location of the body. The odours produced by bodies that are located indoors may not penetrate to the outdoor environment immediately, which will slow the arrival of early colonisers and hence slow the rate of decomposition compared to when insects are present (Byrd *et al.*, 2010; Anderson, 2011; Sutherland *et al.*, 2013). In some cases no odours will be able to penetrate and no colonisation will take place (Pohjoismäki *et al.*, 2010). 2010).

Seasonal changes also have an effect on the colonisation of insects (Schroeder *et al.*, 2003; Villet, 2011). Temperatures in each season affect the insects that are active, as does the physiological diapause (period of inactivity due to stress) where certain species may become

completely inactive due to prolonged periods of cooling (Villet *et al.*, 2010; Villet and Amendt, 2011).

The aim of this chapter was to present the findings of a series of medico-legal autopsies and the associated insect colonisation in different stages of decomposition, locations and season. This chapter will also highlight the data collected at the death scene concerning weather, temperature and other important ecological information.

2.2 Materials and Methods

2.2.1 Site of study

This study was conducted at the Johannesburg Forensic Pathology Service (FPS) Medico-Legal Laboratories, located in Braamfontein, Gauteng Province, South Africa. The facility's primary function is to conduct medico-legal post-mortem examinations in cases of unnatural deaths (including suicidal, homicidal, accidental, or deaths where circumstances are unknown or questionable) in the Johannesburg area (Figure 2-1).

2.2.2 Geography and Climate of study area

Johannesburg is a mosaic of high-density urban dwellings, informal settlements, low-density urban dwellings, rocky outcrops, open public parks and old mining tailings (mine dumps). This provides a multitude of environments with varying ecologies. Johannesburg forms part of the Highveld with an elevation of 1530m above sea level, and has a combination of Egoli Granite Grassland and Gold Reef Mountain Bushveldt vegetation (Cowling *et al.*, 2004). The area has a mean annual precipitation of 600 to 800 mm. The average summer temperatures range from a minimum of 13^{o}C to a maximum of 34^{o}C, and the average winter temperatures range from a minimum of -1^{o}C to a maximum of 23^{o}C. Seasons were defined using the broad categories from the South African Weather Service (SAWS, 2017) as Autumn from 01 March to 31 May, Winter from 01 June to 31 August, Spring from 01 September to 30 November, and Summer from 01 December to 28/29 February.

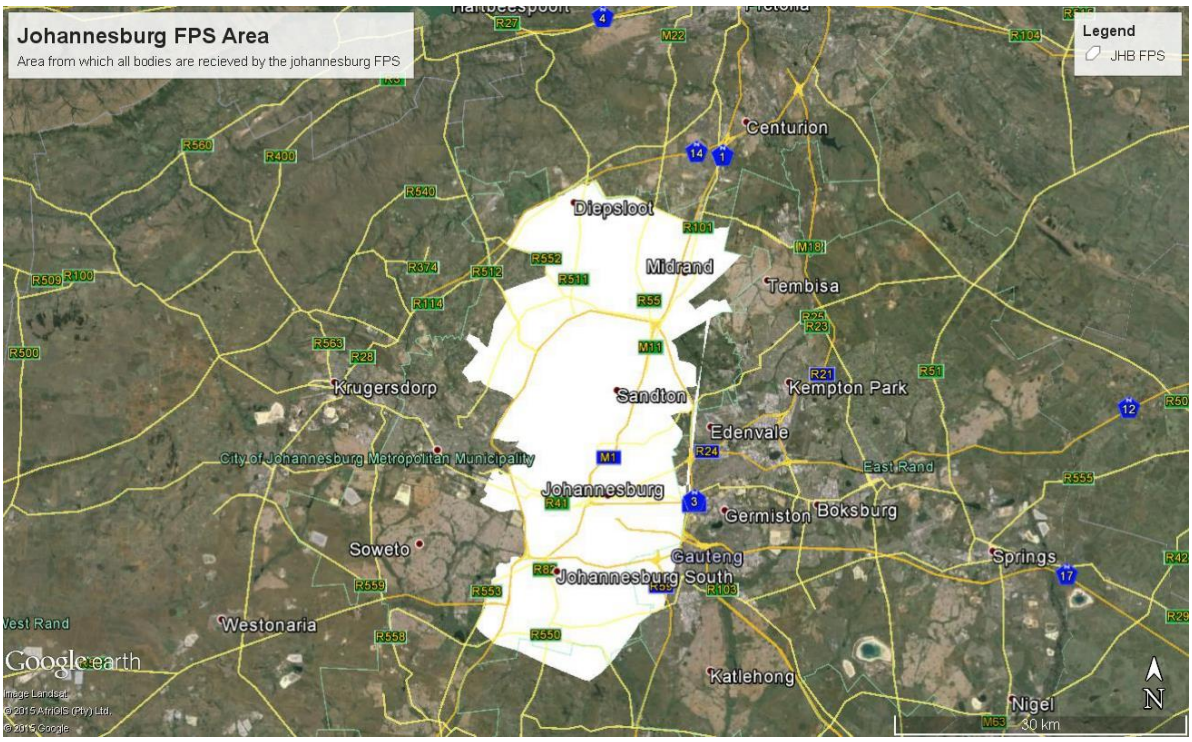


Figure 2-1. Map of the area serviced by the Johannesburg Forensic Pathology Service. The cream white area highlights the area from which bodies are collected. The borders are main roads and highways that separate FPS catchment areas. Map and polygon produced using Google Earth Pro.

2.2.3 Data collection

Examination of bodies

A total of 3,427 medico-legal deaths were autopsied at the Johannesburg FPS between January 2012 and May 2013 (16 months). The bodies had all been removed from the death scene after the police had collected evidence. The bodies were not placed in body bags during transportation in the FPS vehicle because body bags were not available as a result of administrative and operational issues in tenders and procurement. Vehicles were not temperature regulated. At the mortuary, bodies were stored in a refrigerator set to 5^{o}C until the time of autopsy. Autopsy sessions were performed Monday to Friday mornings with four bodies being allocated to each of four forensic medical practitioners (16 bodies were autopsied per day). Allocations were made based on the number of bodies present where an excess of bodies would be carried to the following day.

Data were collected from bodies with insect activity present or signs of past insect activity (i.e. pupal casings and insect castings). Bodies in all stages of decomposition were sampled. For the purposes of this study, the bodies were classified into one of six stages of decomposition:

Fresh stage (from onset of death to initial signs of discoloration),

Early stage (from initial signs of discoloration to bloating, pre-bloat stage),

Bloat stage (from initial bloating until all signs of bloating had disappeared),

Active decay stage (from post-bloat until removal of majority of flesh leaving only dried flesh),

Dry stage (only dried flesh and hair remain),

Skeletal (all flesh having been removed).

Data were collected from the decomposed bodies during medico-legal autopsies with the consent of the forensic pathologist performing the autopsy. Data collection began after the forensic pathologists had completed their external analysis of the body and the clothes removed. Once the items of clothing had been removed these were thoroughly checked for insects or their puparia and castings (pupal skins remaining after adults have emerged). The natural orifices of the body were carefully checked for eggs and larvae as these are the most common sites of insect feeding activity (Smith, 1986). Wound sites, skin folds and genitals were carefully investigated for the presence of insects.

A minimum of 10 to 30 individuals of each insect species were collected from each body consisting of any life stage (egg, larva, pupa, or adult). Egg and larval samples were placed on chicken livers and reared to the adult stage at room temperature (±24^{o}C). Larvae reference samples were killed within one hour of collection in boiling water and placed in 70% alcohol. Collected adult insects were killed with ethyl acetate and then pinned. All samples were identified using morphological taxonomic keys. Identification to family level of the Diptera was performed using the key of Smith (1986: 68-73), and to the genus level using the keys of Smith (1986: 102-104, 109, 116-117) and those of Zumpt (1965:11-16) and Zumpt (1972: 50-64). Identifications of the Calliphoridae and Sacrophagidae were additionally confirmed using the key of Brink (2009). All Coleoptera were identified to family level using the descriptions of Smith (1986:138-149).

Scene information from each case was obtained from the SAP180 (South African Police Death Scene Form) and FPS scene form, which included the scene type (indoor, outdoor or buried), the clothing present, the external manner of death, time of year (season) and any environmental conditions noted. The forensic pathologist autopsy report, in conjunction with visual notes taken by the primary researcher, were used to record the stage of decomposition

and the final cause of death (the specific physiological condition that lead to the demise of the person).

2.2.4 Ethics

Ethical clearance for this study was granted unconditionally through the Wits University Human Research Ethics Committee (Medical). Clearance Certificate M170781 (previously M110835 but a request for an extension was made to ensure current ethics clearance for the purposes of submitting this dissertation).

2.2.5 Data Analysis

Diversity and evenness

Insect species diversity and evenness (see Begon *et al.* 1996) was determined using the Shannon Index for species diversity. The Shannon Index of diversity (H) is calculated using the equation:

$$H = -\sum_{i=1}^{n} pi \ln pi$$

Where the proportion of the species is calculated relative to total species number (pi) and multiplied by the natural log of this proportion (ln pi).

The Shannon equitability or Evenness (E_H) is calculated using the equation:

$$E_H = \frac{H}{H_{max}} = \frac{H}{lnS}$$

Where H is the Shannon Index and H_{max} or lnS is the maximum H for that species or the natural log of the species richness.

Statistical analyses

Data analysis was performed using SAS Enterprise Guide 9.1. Chi-squared analyses were performed to compare the observed versus the expected proportions of the number of bodies received with insect colonisation with the number per season, per month, and for each stage of decomposition. Chi-squared tests for association were performed on: the stage of

decomposition and associated taxa; the environment from which the body was found (indoor vs outdoor); and the number of bodies for each season.

A Kruskal Wallis test was performed to determine the difference between the stages of decomposition for each season. For the purpose of this analysis the stages of decomposition were treated as ordinal data. Stages of decomposition were coded as dummy variables from one to six based on their order in progression on the scale.

Logistic regression on the temperature, stage of decomposition, scene (indoor vs outdoor), and season with the insect species as the independent variable was done. Backward stepwise regression was done to exclude factors which were not significant to the model prediction of species colonisation. Linear regression on the temperature and the coded dummy variables for the ordinal decomposition stages with the number of insect taxa as the independent variable.

A 5% level of significance was used for all statistical analyses.

2.3 Results

2.3.1 Decomposition

Of the 3,427 cases received by the mortuary during the sampling period, 97 (2.83%) cases were in various stages of decomposition (all the others were fresh cadavers with no signs of insect colonization). Of the 97 decomposed bodies, 33 (34.02%) were found to have insect activity present.

Bodies with insect activity that were classified as "fresh" (before the onset of discoloration), comprised the largest portion of the sample compared to that of bodies undergoing decomposition (Figure 2-2). Decomposed bodies (not including burnt bodies) as a whole comprised 66.67% of the sample (n=33).

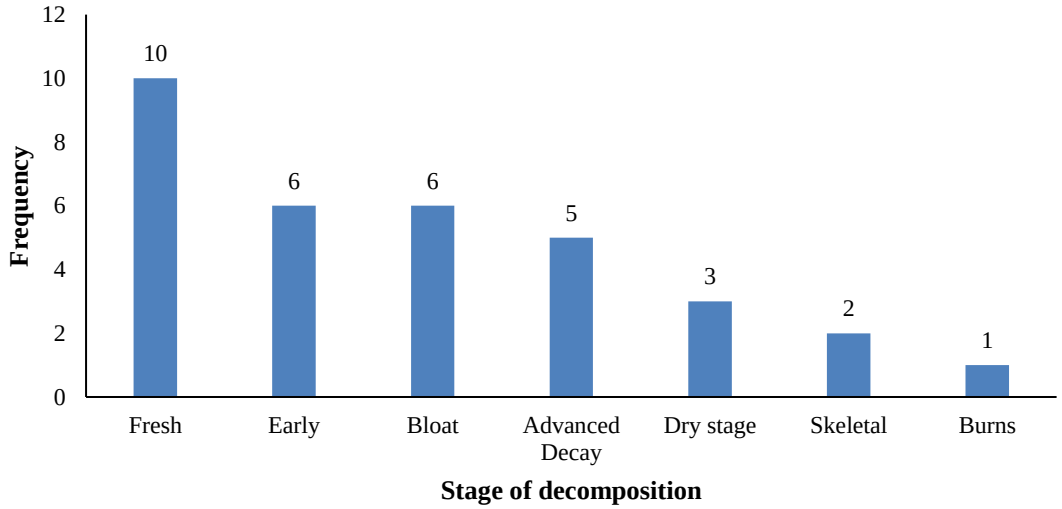


Figure 2-2. Total number (Frequency, y-axis) of decomposed bodies (n=33) with insect activity for each stage of decomposition (x-axis). The single burns case had significant charring and stage of decomposition could not be determined.

2.3.2 Demographics of bodies

There were 25 male cadavers and only six females. Two cases had insufficient information to determine the sex of the deceased, as both these cases were skeletal juveniles.

The ages of the bodies, as determined from identity documents or morphometric estimation, ranged from foetal to 87 years. The mean age was 43.5 (SE = 4.55) with a median of 45. Males ranged from foetal to 69 years with a mean age of 41.9 (SE = 4.78) and a median of 40. Females ranged from 28 to 87 years of age with a mean of 53.8 (SE = 9.88) and a median of 62.5. Females showed the greatest skewness in the age distribution but only six cases were identified in the 16 month period with insect activity present. The remaining unknown cases were those which required anthropological analysis (age estimation using the skeleton and developmental changes to the skeleton to estimate age). No accurate estimation of age was given because of the condition of the remains.

2.3.3 Identification

Twenty-one bodies were positively identified during the course of the study. These were confirmed using visual identification and identity documents of the deceased. Twelve bodies remained unidentified: three were sent for finger print identification; five had DNA taken

from either the head of a femur or muscle; and the remaining four were too badly decomposed for samples to be taken for identification (Figure 2-3). Bodies found in the earlier stages of decomposition were more easily identified. Bodies in the dry and skeletal stages remained unidentified as a result of decomposition and even DNA identification had failed to assist in identification.

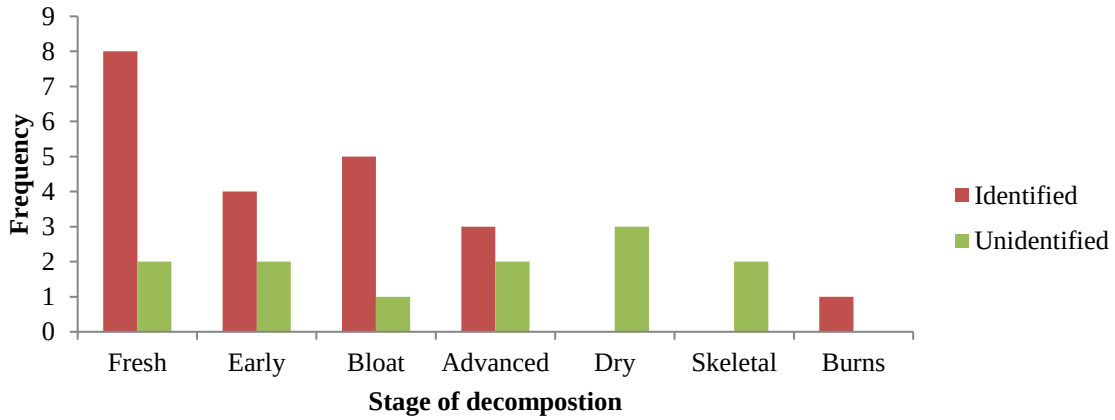


Figure 2-3. Frequency of bodies analysed with insect colonisation (y-axis) at each stage of decomposition (x-axis) in relation to the identification of the deceased.

2.3.4 Cause of death

Thirteen cases (39.39%) had a definitive cause of death determined, while 20 cases (60.61%) had no cause of death determined (Table 2-4). Of those with no cause of death, four were still awaiting results of toxicology and histology analyses (under investigation at the time that data were collected for this study[1]). Almost half (16/33, 48.48%) of the cases were so badly decomposed with autolysed organs that no cause of death was ascertained.

All fresh bodies (n=10; 30.30%) had a known cause of death. As the stage of decomposition increased the number of unascertained causes of death also increased. Dry stage and skeletal stage cases had no ascertained cause of death because of the loss of tissue.

[1] A seven year backlog in such cases means that toxicology results are often outstanding.

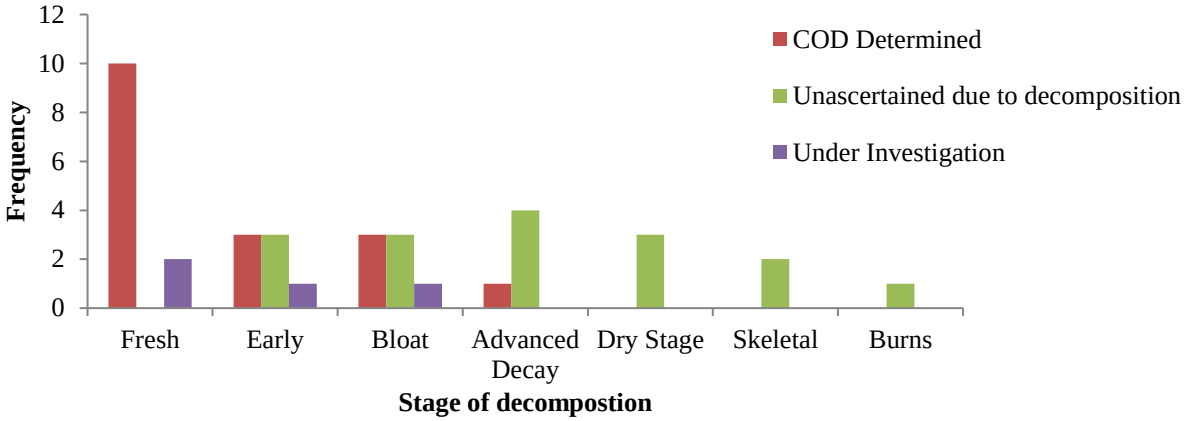


Figure 2-4. Bodies with insect colonisation (y-axis) in relation to the stage of decomposition (x-axis) indicating the ability of the forensic pathologist to determine the cause of death. Bodies listed as 'Under Investigation' were still awaiting toxicology, histology or anthropology reports to determine the cause death.

2.3.5 Location of body with insect colonisation

Eighteen bodies (54.55%) were from indoor environments and 14 (42.42%) were from outdoors/open environments. One case (3.03%) had been buried.

Seven bodies from indoor environments were in the fresh stage of decomposition and five occurred in the early stage (Figure 2-5). A Kruskal-Wallis test found no significant difference between the stage of decomposition and the location (indoor vs outdoor) (χ =3.4557; p=0.1450). The remaining six indoor cases were in the bloat, active decay and dry stages. The highest numbers of outdoor cases occurred in the fresh, bloat and advanced stages.

Fifteen indoor cases (77.78%) were from private residences, two (11.11%) were from medical facilities, and one (5.56%) was from a motor vehicle. Seven outdoor cases (50.00%) were found on open fields or veldt, four (28.57%) were found on or next to the road, and three (21.43%) in water (rivers and dams).

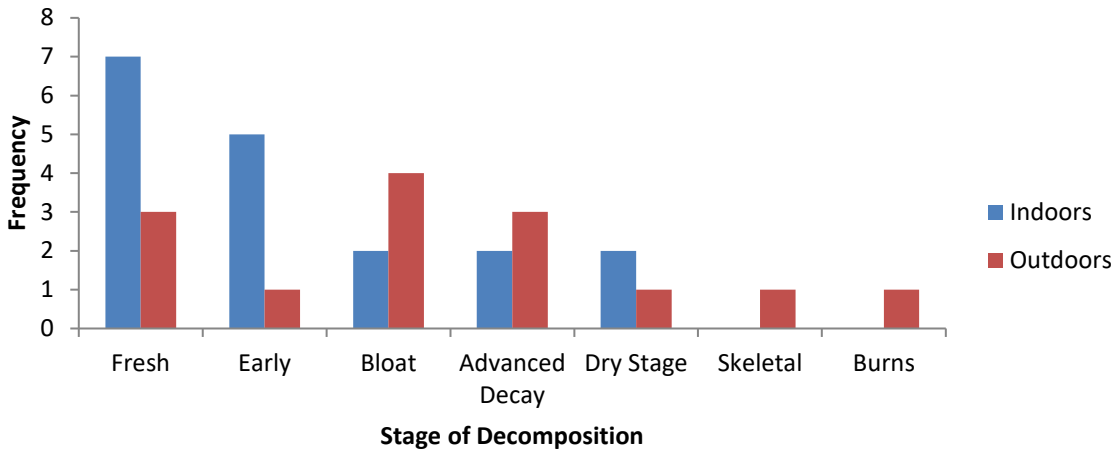


Figure 2-5. The frequency of bodies with insect colonisation (y-axis) found in different environments with relation to the stage of decomposition of the bodies (x-axis).

2.3.6 Time of year and season

The highest numbers of bodies with insect colonisation were observed in October 2012 and January 2013 (Figure 2-6). During the 2012 period, both February and March yielded no cases of bodies with insects, however, in 2013 both those months yielded two and three cases respectively. The month of May yielded no cases in both 2012 and 2013. A chi-squared analysis found no significant difference between the observed and expected equal number of cases per month ($p=0.9719$, $df.=11$, $\chi=3.9309$).

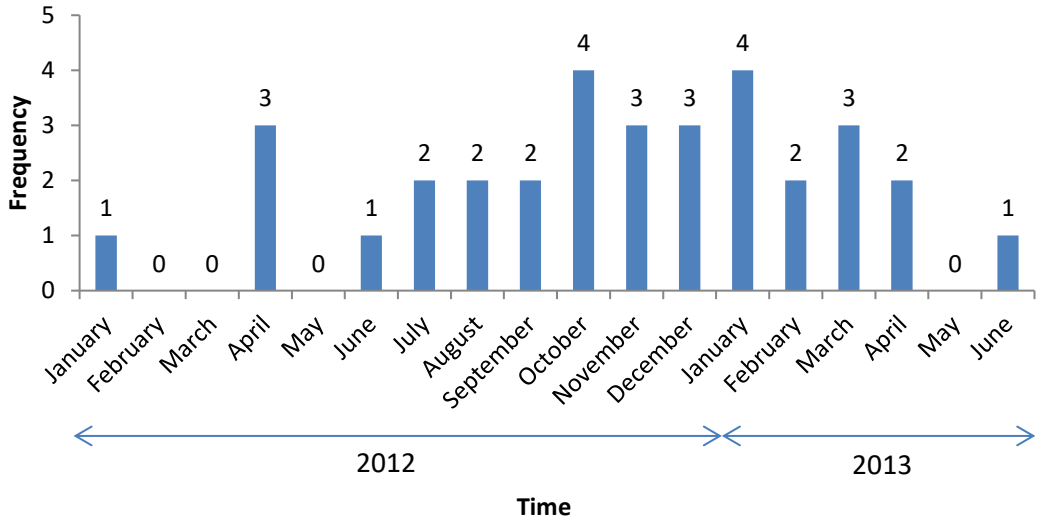


Figure 2-6. Frequency of cases received each month during the sample period. The initial six months only yielded five in total.

The seasonal activity for 2012 (Figure 2-7) showed the greatest number of cases were received during spring. In 2013 an equal number of cases were received from both summer and autumn (n=6 respectively) (Figure 2-8). Both the summer and autumn proportions increased when including the data from both years. A chi-squared analysis of the observed number compared to an equal proportion per season showed no significant difference (p=0.8062, df.=3, χ=0.9795). A Kruskal-Wallis test comparing the stage of decomposition and season also found no significant difference (χ=7.5296, p=0.0568).

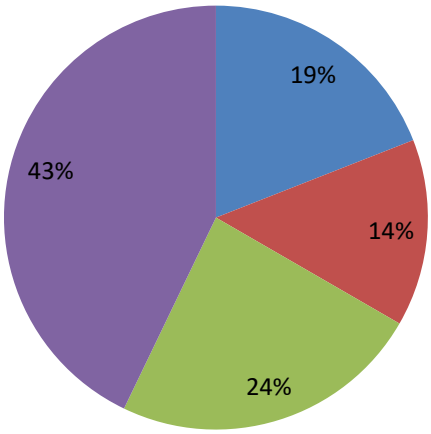


Figure 2-7. The proportion of bodies with insect colonisation received over a full year. Only the first 12 months of the 16 month sample period were used to prevent the seasons being mis-represented.

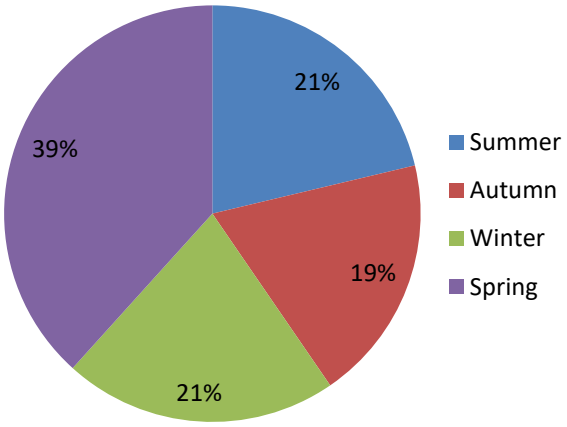


Figure 2-8. The proportion of bodies with insect colonisation received over the whole 16 month sample period, with the averages used for January to June of 2012 plus 2013.

2.3.7 Insect species and influencing factors

A total of fifteen insect species from four orders were collected and identified from human remains (Table I – Appendix 1). The fifteen insect species were collected from bodies in various stages of decomposition. The greatest number of species was found in the active decay stage (n=9) with the early and dry stages having the second greatest (n=5). There was a significant association between the stages of decomposition and the taxa collected from those bodies (χ^2=112.1888, d.f.=84, p=0.0217) (Table 2-1). During the fresh stage *Lucilia sericata* was the most abundant species followed by *Piophilia casei.* Six bodies were received in the

early stage where *Chrysomya marginalis* and *L. sericata* were the most abundant species. *Calliphora vicina* was the most abundant species of the bloat stage. The active decay stage had the greatest number of species present with nine different species. *Chrysomya chloropyga* was the most abundant species of the active decay stage. The dry stage had the second highest number of species, where *Calliphora vicina* was present in the greatest numbers and occurred in all cases in the dry stage. The two skeletal cases sampled had only two species *Ch. albiceps* and a single Histeridae species. The single burn case, which sustained charring to 100% of the body surface, had five different species present (*Ch. marginalis*, *P. casei*. Coleoptera: Cleridae, Staphylinidae and Hymenoptera: Formicidae).

Table 2-1. Insect taxa collected from human bodies (n=33) in various stages of decomposition between January 2012 and July 2013. Each column represents the total number of bodies from which each taxa was collected for each stage of decomposition.

Species	**Total no. bodies**	**Fresh**	**Early**	**Bloat**	**Advanced Decay**	**Dry Stage**	**Skeletal**	**Burn**
Calliphora vicina	10	2	0	4	1	3	0	0
Chrysomya albiceps	8	1	1	0	2	2	2	0
Chrysomya chloropyga	8	0	1	1	4	2	0	0
Chrysomya marginalis	7	0	2	2	2	0	0	1
Lucilia sericata	8	5	2	0	1	0	0	0
Musca domestica	2	0	1	0	0	1	0	0
Nemopoda sp.	1	0	0	0	0	1	0	0
Piophila casei	8	3	1	0	1	2	0	1
Sarcophaga cruentata	1	0	0	1	0	0	0	0
Pediculus humanus var. corpus	1	1	0	0	0	0	0	0
Formicidae	1	0	0	0	0	0	0	1
Cleridae	2	0	0	0	1	0	0	1
Dermestidae	1	0	0	0	1	0	0	0
Histeridae	1	0	0	0	0	0	1	0
Staphylinidae	4	0	0	0	2	1	0	1
Number of taxa per decomposition stage		5	6	4	9	7	2	5

2.3.8 Modelling colonisation

Colonisation by different taxa was analysed using a stepwise logistic regression. Only the average temperature was significant for the model (Wald χ =4.1053, p=0.0427) in predicting the presence of different insect taxa. The number of taxa present in each case was analysed using a linear regression. The temperature (t=2.24; p=0.0326) as well as the stage of decomposition (t=2.66; p=0.0124) were found to be significant predictors in the model (F=4.26, p=0.0235). When grouping the analysis for season, only autumn was significant for both temperature and stage of decomposition (Table 2-2).

Table 2-2. Linear regression model grouped by season with the number of taxa on each body as the dependent variable, and temperature and stage of decomposition as the independent variables.

Season	Variables	Statistic	p-value	Significant
Autumn	Temperature	t= 4,50	0,0064	*
	Decomposition	t= 4,80	0,0049	*
	Model	F= 14,42	0,0084	*
Spring	Temperature	t= 1,41	0,2073	.
	Decomposition	t= 2,24	0,0659	.
	Model	F= 4,63	0,0609	.
Summer	Temperature	t= 0,03	0,9739	.
	Decomposition	t= 1,00	0,3526	.
	Model	F= 0,54	0,607	.
Winter	Temperature	t= 0,09	0,9365	.
	Decomposition	t= 0,51	0,6437	.
	Model	F= 0,21	0,822	.

2.4 Discussion

This study presents the findings of the first analysis of bodies with insect colonisation received at the Johannesburg Forensic Pathology Service mortuary in South Africa.

2.4.1 Decomposition

Decomposed human remains made up only a small percentage of the total number of cases received in the mortuary (2.83% of 3,427 cases). This finding is similar to that of Byard *et al.* (2008), where decomposed bodies made up 6.3% of the 629 cases received for autopsies, and Ambade *et al.* (2011), who recorded 3.6% of 4,997 cases.

The two previously mentioned studies did not however look at the stage of decomposition but rather classified bodies according to presence or absence of decomposition. Keyes *et al.* (2016) performed a retrospective analysis of the decomposed cases received during 2010-2011 at the same facility as this study. They found that 2.24% of all the 4,876 cases received were decomposed: 49.5% were in the early stages, 32.1% were in the bloated stage, 11.9% in the active decay, 2.8% in the dry stage and 3.7% were skeletal. Some of these findings coincide with those of this study where 48.48% of the bodies were in the early stage (fresh and early stages of this study), 18.18% were in the bloat stage, 15.18% in the active decay stage, 9.09% in the dry stage and 6.06% were skeletal (n=33) (the remaining 2.69% were burned cases not classified into a stage of decomposition). The greater number of cases may have been a result of the slightly different definitions in the stages of decomposition used, but temporal differences may be an alternative reason for such a difference. Keyes *et al.* (2016) also found insect colonisation in 25.7% of the decomposed cases, which coincides with the findings of this study where insect activity occurred in 23.7% of all decomposed cases.

Insect activity on decomposed bodies made up a small proportion of all the bodies received at the facility (0.96% of the total number). The lack of insect colonisation on some decomposed bodies at the time of sampling may be because they were not present on the body itself, but were rather beneath or around the body at the death scene and as a result were not collected before the body was removed from the death scene (Byard *et al.*, 2008). The inability of the insects to gain access to the body (Hall and Huntington, 2010), or the loss of insects as a result of movement of the body between the death scene and the mortuary may also have contributed. The latter would not normally come into consideration because body bags are usually used. However, at the time of sampling for this study, body bags were not available at the facility. Bodies were placed onto mortuary trolleys and transported in the mortuary vehicle where up to four bodies could be transported together. The potential for insects to

colonise other bodies during transportation cannot be excluded and may have affected some cases recorded.

This study found that the earlier stages of decomposition made up the greatest proportion of insect colonisation with respect to the number of bodies received rather than later stages of decomposition. The later stages of decomposition were, however found to have greater numbers of taxa present.

2.4.2 Sex and Age

Males made up 75.76% of the decomposed bodies with insect activity. Male predominance in medicolegal studies is relatively common (Byard *et al.,* 2008; Ambade *et al.,* 2011). It has been suggested that the possible predominance of decomposed males is the social isolation and a lack of healthcare with increased age, resulting in death where the bodies are often not located for some period of time (Byard *et al.,* 2008; Ambade *et al.*, 2011). The present study also found male predominance mainly in two age categories: 31 to 40 and 61 to 70. There were also a large number of males in the "unknown" age category where no age was determined at the time of sampling. Female predominance was only seen in the 71+ years category, which has been reported to be the age of greatest risk for elderly females who are isolated or alone and may be susceptible to injury or attack (Taylor and Ford, 1983).

2.4.3 Identification

Decomposition and other post-mortem changes, including insect removal of soft tissue, may confound the identification of the body by altering the features of the deceased, making visual identification impossible and requiring alternative techniques to be used (Payne-James *et al.,* 2011). In the present study, only 63.63% of the bodies were identified and almost all were in the early stages of decomposition (fresh to bloat) when the visual features (facial, tattoos, scars and hair) are mostly intact (Saukko and Knight, 2004). Insect samples in the present study were mainly collected from the natural orifices of the head during the fresh stages of decomposition, but in all areas of the body in the later stages of decomposition. All of the positively identified cases were identified visually by relatives of the deceased. The remaining cases were sent for fingerprint identification, DNA profiling and anthropological analysis

(demographic determination from skeletal measurements) however none have been positively identified. This is not uncommon, as very few badly decomposed cases are ever positively identified (personal communication – mortuary manager).

2.4.4 Cause of death

Determination of the cause of death is the primary goal of all medico-legal autopsies. However, decomposition and putrefaction of the remains can make determining the cause of death increasingly difficult as decomposition progresses (Saukko and Knight, 2004). In the present study all bodies in the fresh stages of decomposition (n = 10) had a cause of death which could be determined. The number of bodies with a cause of death decreased with the advancement of decomposition. Only 39.4% of the 33 bodies in this study had a cause of death which could be determined. This is in direct contrast to that of Ambade *et al.* (2011) and Byard *et al.* (2008) where both studies found that cause of death was determined in more than 85% of cases. Possible explanations for this difference are that the present study focused not only on decomposed cases but specifically decomposed cases with insect activity. Insects, especially maggots are the main removers of tissue (Saukko and Knight, 2004), which may have diminished the quality of tissue present at autopsy and hence the ability of the pathologist to determine the cause of death.

A number of cases in the present study had no cause of death determined because of outstanding toxicological results. This is a national problem in South Africa with toxicological results outstanding for several years after the death of an individual (Sifile, 2016).

2.4.5 Location of the body

More than half of the cases in this study were found in enclosed or indoor environments, the majority of which were in the fresh stage of decomposition. This number gradually decreased with the advancement in the stages of decomposition of all other cases. These indoor bodies would have decomposed at a slower rate through more moderate and stable temperatures, and limited access by insects (Anderson, 2011).

The outdoor cases, however, had two distinct peaks in their numbers: one in the fresh stage and one in the bloat stage. The high number of fresh stage cases may have occurred through bodies being located shortly after death. The second peak in numbers in the bloat stage is suspected to have occurred due to the escaping of putrefactive gases that would have attracted both insects and people to the site of the remains.

It is well established that outdoor carcasses are more easily colonised by insects than indoor carcasses, resulting in more species colonising outdoor remains (Schroeder *et al.*, 2003; Anderson, 2011). Colonisation by insects is not inhibited outdoors (unless the body is wrapped in cloth or plastic) allowing easy detection of chemical cues (Leblanc and Logan, 2010; Simmons *et al.*, 2010). *Musca domestica* was only collected from indoors bodies, presumably because of the highly synanthropic nature of the species and its tendency to be found within human dwellings, being attracted to food, refuse and dead flesh (Smith, 1986). *Lucilia sericata* also occurred in high numbers indoors, being a species that is more associated with the flesh of humans and may be involved in feeding on both living and dead flesh (Zumpt, 1965; Smith, 1986; Pohjoismäki *et al.*, 2010). Only dipteran taxa were collected from indoor bodies while coleopteran species were found on bodies from outdoor environments. This finding agrees with that of Anderson (2010) where no beetle species entered the indoor environment for the entire duration of his study. Buried bodies in this study had the lowest species diversity, which agrees with the findings of Simmons *et al.* (2010a) where burial acts as a physical barrier to colonisation, preventing insects from accessing the body. Location of a body has been reported by many authors to have a considerable effect on the ability of insects to colonise it (Goff, 1993; Pohjoismäki *et al.*, 2010; Simmons *et al.*, 2010a). In the present study, location was not significant in determining either the taxa present or the numbers found, suggesting that access to the body is more important for the physical presence of insects rather than the taxa.

2.4.6 Time of year and season

Insect colonisation was consistent throughout the year. May was the only month where no cases with colonisation were received in both years. Time of year and season were found not to be significant in the occurrence of different insect species and in the abundance of species. The changing seasons and time of year may only be important with regards to the temperature

(which was found to be significant) and there may have been an initial lag phase in insect colonisation or decrease in insect numbers as a result of the decrease in temperature in May.

Typically, warmer seasons have been observed to have more insect colonisation than the cooler seasons (Schroeder *et al.*, 2003), which correlates with the findings of the present study, where temperature was found to be a significant factor in both the species diversity and the numbers present. This seasonal variation was also noted by Byard *et al.* (2008) in their study, however a larger proportion of the cases sampled then were found in the summer months (as in Schroeder *et al.* (2003)) compared to the larger spring sample in the present study. A study by Bharti and Singh (2003) in India on rabbit carcasses, found spring to have the highest number of species present and agrees with the findings of this study. Bharti and Singh (2003) suggested that a possible reason for the high number in spring is the more moderate temperatures experienced by the insect colonisers that may support the combined abundance of both late winter and early summer insects. Kelly (2006), in a study conducted in central South Africa, found that autumn and winter had the greatest insect abundance, and Bharti and Singh (2003) found that autumn had the second highest abundance. The present study found that when observing the effects of temperature and decomposition on the number of taxa occurring, autumn was the only season with a significant result suggesting that the numbers collected in autumn was a result of the temperature and the decomposition together. This finding suggests that temperature and decomposition play a greater role than the season in the abundance of insects which may be a result of the subjective definition of season used for convenience.

2.4.7 Insect species and influencing factors

Fifteen insect taxa were collected and identified throughout the duration of this study. Thirteen of these are regarded as forensically important in the determination of the PMI as they have strong associations with decomposing bodies, the Dipteran and Coleopteran families being the main taxa (Smith, 1986). The remaining taxa were those of the Families Formicidae (which are common soil inhabitants that will colonize bodies in plant litter or on soil surfaces) (Smith, 1986), and Pediculidae (*Pediculus humanus* var. *corpus*, which is temperature sensitive and will leave the body or die after three days (Smith, 1986; Gennard, 2016) and may therefore be a very important tool in death investigations).

Calliphoridae were the most abundant during the fresh and early stages of decomposition with *Lucilia sericata* being the dominant species. In studies of the colonisation of pig carcasses, *L. sericata* was found to be immediately attracted to the carcass, laying eggs shortly after arrival (Anderson, 2011). *Calliphora vicina* occurred in the fresh stage but was the most abundant species of the bloat stage and again in the dry stage. Even though the species has been sampled in early stages of decomposition (Anderson, 2011) it has also been noted to occur in later stages (Erzinclioglu, 1996).

The active decay stage, typically with more taxa than other stages (Smith, 1986; Goff, 2009; Léblanc and Logan, 2010), had the greatest diversity here too, with nine different taxa present. Dipterans were the most common organisms during this stage and are often attracted to a body in great numbers as a result of the release of putrefactive gases (Goff, 2009). Even when the number of bodies sampled is low, the numbers of taxa present on remains in the active decay stage is consistently high, supporting the strong link between the active decay stage and species attendance. *Chrysomya chloropyga* was the most common species in this stage. In a study by Kelly *et al.* (2009), they found *Ch . chloropyga* also during the dry stage but only in their autumn sample and *Ch. marginalis* was the dominant species present.

The dry stage, in the present study, had the second highest number of species where, as mentioned, *Ca. vicina* was the most abundant occurring in all cases received in the dry stage. *Chrysomya albiceps* and a single Histeridae species were found during the skeletal stage, with *Ch. albiceps* only present in the pupal stage where they emerged from the puparia the day after collection. The single burns case had five different taxa present (Diptera: *Ch. marginalis*, *P. casei*. Coleoptera: Cleridae, Staphylinidae. Formicidae sp.), which was the second highest number of taxa received on a single case. This may be a result of the alteration of the body as a result of burning, causing the body to artificially appear in a more advanced stage of decomposition (Gruenthal *et al.*, 2012), and hence attract later colonizers in conjunction with early colonizers.

2.4.8 Modelling colonisation

This study found that temperature was a significant factor in determining the taxa present on a decomposing body. Even though decomposition was used in the stepwise logistic regression, it was not significant in determining the taxa present on the body. Decomposition is an important factor for colonisation (Smith, 1986; Archer and Elgar, 2003; Anderson, 2011), but

the current study found temperature to be the most important factor. George *et al.* (2013) found temperature was a significant variable in predicting the presence of colonisation, which correlates with the findings of this study. The lack of significance of other variables (location and season) suggest that the complex relationship between abiotic variables and insect colonisation requires more research to better understand the factors and their interactions. The small sample size of the current study cannot be excluded as a possible contributing factor.

2.5 Conclusion

Insect colonisation is known to be an important tool in forensic investigations (Amendt *et al.*, 2007). While the number of cases with insect colonisation used in this study was relatively small compared to the total number of cases received by the mortuary, the importance of insects becomes most evident in cases where decomposition has advanced beyond the point where forensic pathologists can determine a PMI (Payne-James *et al.*, 2011).

Insects may also be a confounding factor in relation to determining the cause of death, through accelerating the decomposition of human remains and making the interpretation of post-mortem findings more difficult. While insect association with the stage of decomposition has been well researched on pig carcasses, there is still great value to be gained from human case studies in mortuaries. Most studies conducted in the mortuary are by forensic pathologists, with very few studies from anthropologists or entomologists in this setting. While fully controlled studies are essential to develop better techniques in the determination of the cause of death, identification of the body and estimation of the PMI, there is still a wealth of knowledge available from the findings, or lack of findings in many cases, of autopsies.

CHAPTER THREE

Modelling Temperature Variations from the Death Scene to the Time of Insect Collection at Autopsy: Implications for Estimation of the Post-Mortem Interval

3.1 Introduction

The positive association of insect development/age with temperature is well established (Amendt *et al.*, 2007; Harvey *et al.*, 2016). It is this association that allows for insect development to be used in the estimation of time since colonisation in bodies where time of death is unknown (Goff, 1993; Gennard, 2007). This estimation of time since colonisation provides a minimum post-mortem interval (PMI) that can be used to infer the time of death (Gennard, 2007). This time of death estimation is especially important when circumstances surrounding the death are unknown. As the time since death increases, the importance of estimates using insect colonisers becomes increasingly important as other methods of PMI estimation become less accurate (Goff, 1993).

3.1.1 Methods of PMI estimation

There are a number of methods used for the estimation of the PMI in forensic investigations (see Sharma *et al.* (2015) and only the two most commonly used ones are discussed here. The first method uses the developmental length of the insect larva as a function of temperature and time to produce diagrams known as isomegalens or isomorphens (Grassberger and Reiter, 2001). This method provides a quick and easy reference to estimate the temperature dependent development based on the length of a larva to estimate larval age, but it is dependent on a constant average temperature to be accurate (Grassberger and Reiter, 2001; Reibe *et al.*, 2010).

The second method is the use of accumulated degree-days (ADD) or hours (ADH). This method utilizes the physiological energy budget required for development with an assumed linear regression which predicts development based on different temperatures over the developmental history of the larva (Gennard, 2007; Harvey *et al.*, 2016). The average

development per hour or per day are added together to provide an accumulated effect of temperature and time and is thus also known as the thermal summation model (Harvey *et al.*, 2016). While this is a relatively simple method, it has the disadvantage of not being able to account for development at the upper and lower threshold temperatures of insect development (Harvey *et al.*, 2016), which are curvilinear at these upper and lower threshold levels (Reibe *et al.*, 2010). As mentioned, these thresholds are curvilinear (s-shaped) which means that as temperatures approach either threshold the development becomes less linear. The upper threshold is the maximum temperature at which development can continue to occur before further increases would result in mortality. The lower threshold is the minimum temperature at which larvae are able to survive before mortality occurs. This is still the most common method of PMI estimation used and researched even with the degree of error at threshold temperatures (Villet *et al.*, 2010).

3.1.2 Considerations when estimating PMI at autopsy

Often the collection of insect evidence is only done at the autopsy (Johl and Anderson, 1996; Huntington *et al.*, 2007) or even after the autopsy (Thevan *et al.*, 2010). There are a number of factors that must be considered when collections are done at the time of autopsy. Firstly, the temperature of the death scene and the time that the body was removed are essential pieces of information (Harvey *et al.*, 2016). Secondly, information is needed regarding the storage of the body prior to the autopsy (time and temperature within the refrigerator) (Amendt *et al.*, 2007), and lastly, when the body was removed from the refrigerator. The storage of the body (and associated insects) prior to autopsy and subsequent insect collection may have implications for the forensic entomologist and the estimation of a PMI.

The period and temperature of cooling during refrigeration may affect the PMI estimation (Johl and Anderson, 1996; Myskowiak and Doums, 2002; Huntington *et al.*, 2007; Thevan *et al.*, 2010; Villet *et al.*, 2010; Harvey *et al.*, 2016). Myskowiak and Doums (2002) found that a period of cooling could alter the overall development of the maggot through behavioural inactivity as a response to harsh conditions, known as diapause. Diapause is essential to winter survival for many species and may occur during short-term mortuary refrigeration, especially when refrigerator temperatures are below the lower thermal threshold when development would be affected (Higley and Haskell, 2010).

Huntington *et al.* (2007) found that a significantly warmer micro-climate was created when body bags were used, through bacterial activity producing additional heat. This meant that maggots in morgue refrigerators could remain active instead of entering diapause, which would skew results of the PMI because of temperature differences. Thevan *et al.* (2010) also found that within maggot masses, insect development did not cease with refrigeration. This suggests that refrigeration may still allow for some level of development given certain condition (micro-climate and maggot mass formation). Huntington *et al.* (2007) found that although the fridge temperatures were set to 4^{o}C they in fact fluctuated above 5^{o}C, suggesting that even "set" temperatures could not be used as true representations of temperature. Depending on the fluctuations, the temperature could be higher than the lower threshold temperatures allowing some species to be active in spite of refrigeration.

The aim of this study was to provide a simulated model of the effect of temperatures, from the death scene to the refrigerator, and finally at the time of autopsy. The model simulated the estimated PMI using thermal summation for six dipteran species: *Calliphora vicina*, *Chrysomya albiceps*, *Chrysomya chloropyga*, *Lucilia sericata*, *Musca domestica* and *Piophilia casei*. These species were selected for their forensic importance (Smith, 1986), and because they were also collected and identified from bodies received at the facility (see Chapter Two).

3.2 Methods and Materials

3.2.1 Site of Study

This study was conducted at the Johannesburg Forensic Pathology Medico-Legal Laboratories, located in Braamfontein, Johannesburg, South Africa from January 2012 to June 2013 (16 months). The facility's primary function is to conduct medico-legal post-mortem examinations in cases of unnatural deaths (including suicidal, homicidal, accidental, or deaths where circumstances are unknown or questionable) in the greater Johannesburg region. Between 2500 and 3200 autopsies are performed per annum at the facility.

3.2.2 PMI estimation and modelling variation using Monte-Carlo Simulation

The Accumulated Degree Day (ADD or ADH for Hours) or thermal summation method was used (Gennard, 2007; Higley and Haskell, 2010). The formula for ADD is:

$$ADD = T \times (\Theta - \Theta_0)$$

Where T is the developmental time, Θ is the ambient temperature and Θ_0 is the species specific base temperature. The base temperature is the lowest possible temperature at which development can still occur. When the ambient temperature is below the base temperature the ADD becomes zero as development ceases. The base temperature is calculated using the species specific development at a range of temperatures plotted against time. The thermal summation method assumes a straight line relationship which is then extrapolated backwards to the x-intercept, which is the base temperature estimate for that species (Gennard, 2007).

For the purposes of this study the PMI was estimated based on the following hypothetical scenario. Firstly, after removal from the death scene, on average a body will take an hour to be transported to the medico-legal forensic facility. Secondly, the body will then remain refrigerated for 24-hours before being autopsied. Finally, the post-mortem examination (autopsy) should take no longer than four hours to be completed. The assumption is that at the end of this hypothetical scenario, the insect taxa needed to estimate PMI would be given to the entomologist.

The hypothetical scenario described above was used to perform a Monte-Carlo simulation for the hypothetical time-period, and to calculate the ADD estimation to determine the associated best and worst case scenario estimations for six dipteran taxa (Table 3-1).

Table 3-1. Dipteran taxa and their associated base temperatures as determined by previous research. For the purposes of this study and simulation an average base temperature was used to provide an estimation irrespective the of instar stage.

Species	Base Temperature (ºC)	Reference
Calliphora vicina	1,00	Donovan *et al.* (2006)
Chrysomya albiceps	11,29	Salimi *et al.* (2018)
Chrysomya chloropyga	11,44	Richards *et al.* (2009a)
Lucilia sericata	8,83	Roe and Higley (2015)
Musca domestica	11,98	Wang *et al.* (2018)
Piophilia casei	7,29	Russo *et al.* (2006)

Monte-Carlo simulation is a method of analysing unknowns or variations in a system through a number of analytical assumptions based on an input model (Fedra, 1983). The method has been used widely for simulating financial forecasts but has also been used to provide estimations of PMI (Reibe *et al.*, 2010) and to describe variations in estimates (Higley and Haskell, 2010). Monte-Carlo simulation utilises the mean and standard deviation of input variables to provide the unknown variability in such a system. These data are then modelled to fit a normal distribution based on the assumption that the variability should be normally distributed. The data were fitted to an output model, which was used to provide an initial iteration of the model and then replicated multiple times until a sufficient number of iterations had been performed.

For the purpose of this study, the temperatures obtained from the three locations were used as the input data for the model. From these temperatures a mean and standard deviation was obtained for each. The mean and standard deviation were fitted to a normal distribution using the NORM.INV function in Microsoft Excel 2016 for the scene temperatures and the autopsy room temperatures. The refrigerator temperatures were fitted to a uniform distribution. The model variables to describe the variability in the output was obtained using the RAND() function (Microsoft Excel 2016) to provide a random probability for the normal distribution fit, in conjunction with the input mean and standard deviation of the refrigerators. The refrigerator uniform distribution was modelled using the function: minimum temperature + (RAND() x maximum temperature) to obtain a random value within the expected range of the refrigerator. The remaining model variables were those required to estimate PMI using thermal summation from insect development and the base temperature. From these input variables a simulated output was obtained as the first iteration, which was repeated for 1000 iterations to obtain a comprehensive number of simulation replicates.

From the 1000 iterations, proportions of likelihood estimates were obtained for each species based on the no development occurring (i.e. negligible larval development over the three day period) versus development occurring (i.e. development occurring between the time of removal from the scene and subsequent collection after autopsy).

3.2.3 Temperature measurements

Temperature measurements were taken from the external environment, the refrigeration unit and the autopsy suite (Figure 3-1). The temperature of the mortuary refrigerator was set to 4^{o}C (±2^{o}C). Other refrigerators were also in use but these are only for bodies that need to be stored after the autopsies and sample collections have concluded, hence they were excluded

from this study. The actual make and model of the refrigerator could not be determined as multiple repairs and alterations had taken place over the years prior to this study (personal communication with Mortuary Asset Manager).

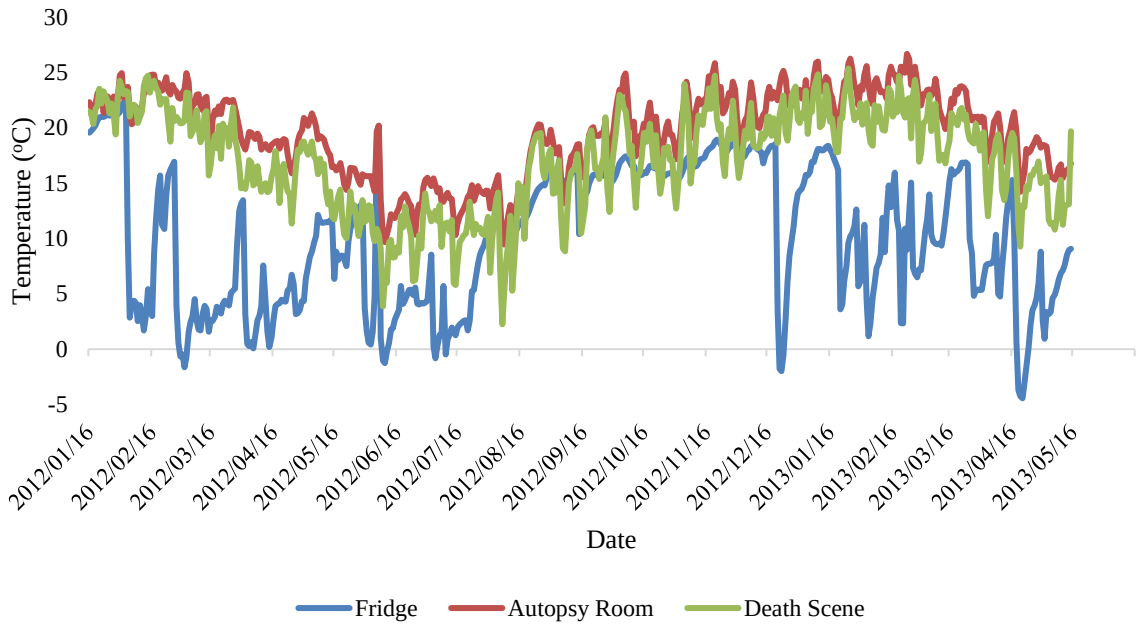


Figure 3-1. Temperature measurements recorded over 16 months. The data generated were used to provide input data for the Monte Carlo simulation. Fridge: $\bar{x}$=10.171, SD=2.958. Autopsy room: $\bar{x}$=19.640, SD=3.846. Death scene: $\bar{x}$=17.133, SD=4.563. The four month period where the Fridge temperatures were excessively high was due to a mechanical failure.

Data on the temperatures of all three locations were collected using Thermocron® iButtons® (Model: DS1922L). Two iButtons® were placed in each location to ensure that a more uniform measure of temperature was obtained for the site. In the refrigeration unit, one iButton® was placed inside near the door and the second iButton® was placed on the opposite wall furthest from the door. The refrigerator had sliding doors that had to be manually closed to maintain temperatures within the room. In the post-mortem room, iButtons® were placed on opposite walls, one near the door and the second on the opposite wall (an air conditioner was present in the room but it was not functional throughout the duration of the study). For the external environment, iButtons® were placed outside the facility in the receiving area of the mortuary that received no direct sunlight. Additional temperature measurements were obtained from the South African Weather Services (SAWS, 2013) to ensure that the readings obtained by the external iButton® was close to the SAWS ambient temperature (accounting for micro-climatic variation).

3.2.4 Data Analysis

SAS Enterprise Guide 9.1 (SAS institute, Cary, NC) was used for all statistical analyses. For the purposes of this study a 95% level of significance ($\alpha = 0.05$) was used. Likelihood of development measures were compared using Pearson Correlation using the base temperature of each species.

3.3 Results

The Monte Carlo simulation data obtained are summarised in Table 3-2 for each of the six dipteran species when insect samples are only collected after the completion of the autopsy. *Calliphora vicina* had the highest mean ADD and maximum ADD of all the species simulated. The minimum values were limited to zero to indicate development ceasing when temperatures were below the base temperatures indicated in Table 3-1.

Table 3-2. Summary statistics of ADD for six forensically important Dipteran species from 1000 iterations of Monte Carlo simulations. All measures are in ADD. Each percentile represents the ADD value at each corresponding point in the distribution (i.e. the 90th percentile represents the ADD value that is greater than 90% of the other ADD values in the distribution).

Dipteran Species	Mean	SD	Min	Max	Percentile						
					5th	10th	Lower Quartile	Median	Upper Quartile	90th	95th
Ca. vicina	13,37	7,42	0,00	37,76	1,80	3,88	7,44	13,15	18,50	23,29	25,98
Ch. albiceps	11,64	7,07	0,00	35,12	0,46	2,26	5,86	11,60	16,82	21,32	23,53
Ch. chloropy	11,45	6,79	0,00	30,10	0,91	2,64	5,75	11,34	16,66	20,38	22,81
L. sericata	11,75	7,01	0,00	35,18	1,12	2,66	6,23	11,18	16,79	21,29	23,98
M. domestica	11,61	6,85	0,00	31,61	1,09	2,59	6,12	11,40	16,66	20,81	23,31
P. casei	12,21	7,08	0,00	36,71	1,42	2,81	6,58	12,05	17,26	21,66	24,64

The distributions of the ADD for all six species were found to be right skewed (Figure 3-1). The skewness obtained is an effect of limiting all the ADD minimum values to zero, in addition to the effects of the simulated uniform distribution of the refrigerator temperatures. The percentile values presented in Table 3-2 provide a more accurate representation of the actual distribution by focusing on the proportion of values rather than the actual value itself.

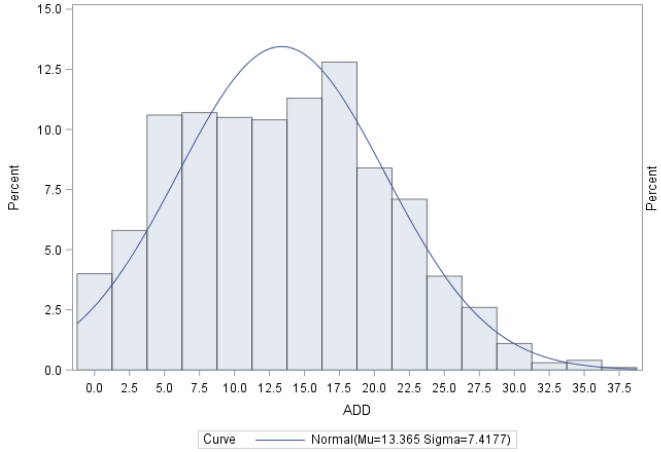
The results presented in Table 3-2 and Figure 3-1 indicate the expected variation in ADD for each species based on the modelled temperature and times, from the time the body is removed from the scene until the collection of insects following refrigeration and autopsy.

The effect of refrigeration alone was modelled separately to provide a better understanding of the effect on development of each species based on the variation in refrigerator temperature above and below the base temperatures of each species (Table 3-1). Table 3-3 provides a summary of the effect on development.

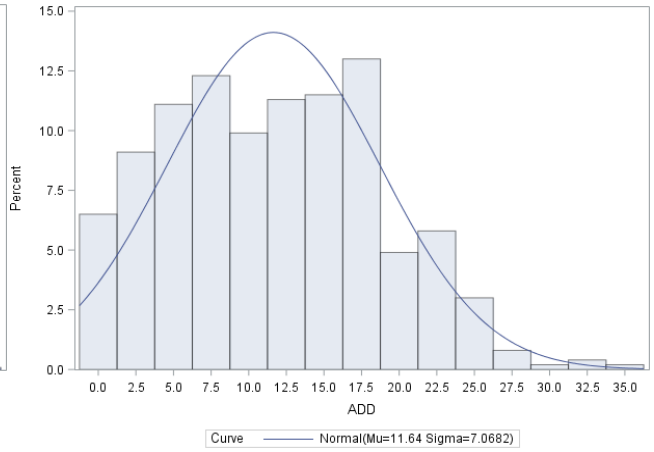
Table 3-3. The expected likelihood of development on six species based on a uniform distribution of a refrigerator set to 4°C, modelled using Monte Carlo simulation.

Species	**Likelihood no development**	**Likelihood continued development**
Calliphora vicina	24.00%	76.00%
Chrysomya albiceps	71.60%	28.40%
Chrysomya chloropyga	70.80%	29.20%
Lucilia sericata	58.10%	41.90%
Musca domestica	71.20%	28.80%
Piophilia casei	56.80%	43.20%

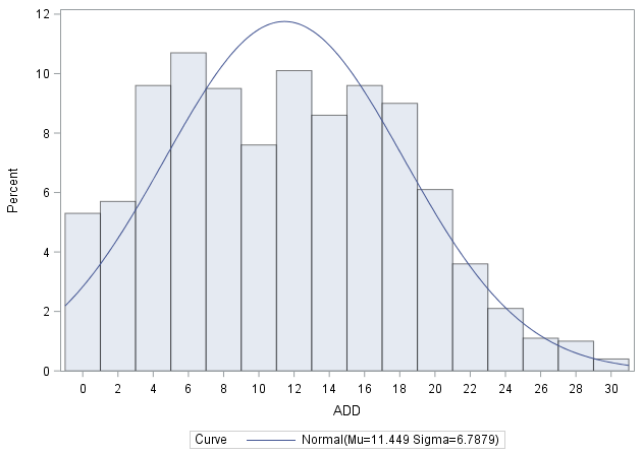
Development during refrigeration varied between the six species. Development likelihood values were found to be negatively correlated with the base temperatures of the species (Pearson Correlation = -0.99, p=0.0001), where species with higher base temperatures (*Ch. albiceps*, *Ch. chloropyga* and *M. domestica*) were less likely to continue development than those with lower base temperatures.



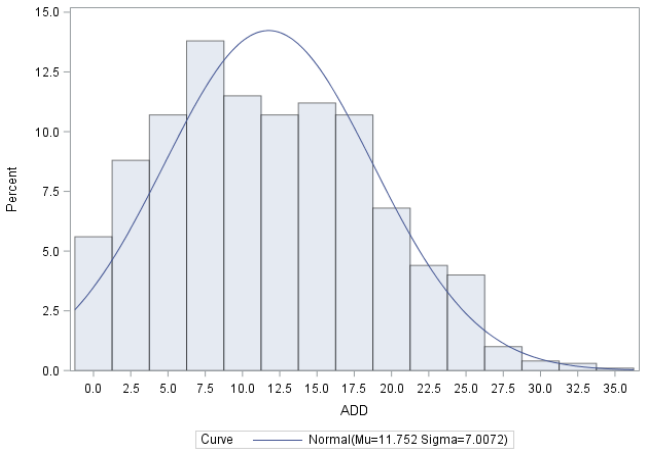
(i) Calliphora vicina



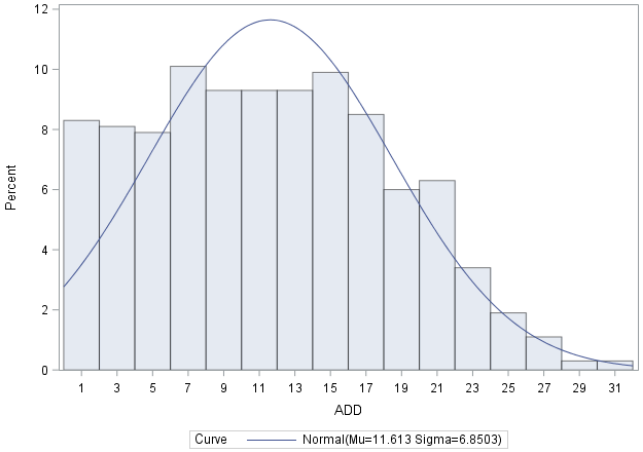
(ii) Chrysomya albiceps



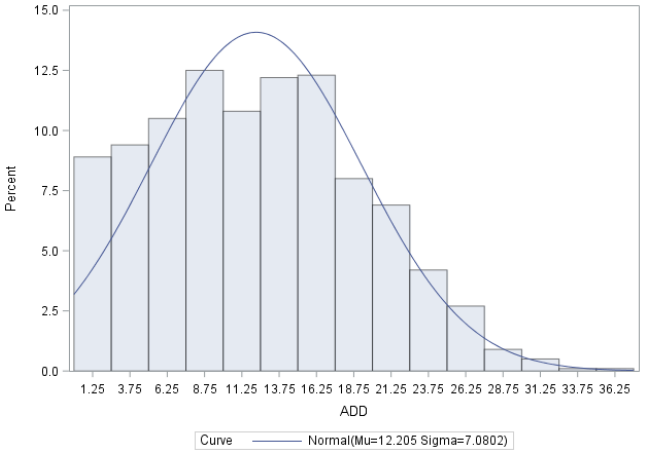
(iii) Chrysomya chloropyga



(iv) Lucilia sericata



(v) Musca domestica



(vi) Piophilia casei

Figure 3-2. Monte Carlo simulation distributions of ADD for six forensically important species.

3.4 Discussion

This study provides a simulation of the uncertainty present when estimating the PMI using the thermal summation method from actual temperature variations recorded over 16 months at a South African mortuary. Prior to the autopsy, all cases that are received are usually placed into refrigerators to prevent further decomposition before examination by the forensic medical practitioners. This has two obvious effects: firstly it slows the rate of decomposition ensuring a more productive autopsy, and secondly it slows the rate of development of any insects present on the remains (Goff, 2009).

A number of studies have analysed the effect of refrigeration on maggot development (Johl and Anderson, 1996; Myskowiak and Doums, 2002) and survival following rapid cooling (Chen *et al.*, 1987; Coulson and Bale, 1990). This study expands on the potential effects of refrigeration through simulating the effect that refrigeration will have on PMI estimation and ADD measures. The simulated time period in this study provides a measure of uncertainty for ADD during transportation, storage and finally the autopsy when insects are collected from the human remains. The ADD values for each species were relatively small, but the effect can result in an increased ADD estimation if thermal summation is used to estimate PMI. This would result in incorrect PMI estimations and potentially misleading time of death estimations.

The skewed distributions of this study were caused by the uniform distribution of refrigeration temperatures used in the simulation and the limiting of the ADD to zero. This further reiterates the warnings of Higley and Haskell (2010), and Villet *et al.* (2010) of the potential issues of estimating PMI when temperatures are close to the lower threshold.

Johl and Anderson (1996) studied the effects of 24-hour storage on the development *Calliphora vicina* where the simulated morgue refrigerator was set to 3^{o}C over a 24 hour period. The authors found variations in development of the larvae when reared after removal from the refrigerator, as a result of the different ages of the larvae. The findings of Johl and Anderson (1996) suggest that the simulation of the present study may still not represent the complete variation that may occur when larval age is also considered.

The simulation model presented in this study highlights the importance of temperature and period of refrigeration on PMI estimation from specific species. The species used in this study include the most common taxa sampled from human cadavers (see Chapter 2), and those which have been studied extensively for PMI estimations (Donovan *et al.*, 2006; Russo *et al.*,

2006; Richards *et al.*, 2009a; Thevan *et al.*, 2010; Roe and Higley, 2015; Salimi *et al.*, 2018; Wang *et al.*, 2018). Based on the findings of the present study, the temperature variations during refrigeration will result in continued development of fly larvae in a large proportion of cases. This highlights the importance for forensic entomologists to carefully consider the insect species present and the species-specific base temperatures especially following cooling of the body. Species with low base temperatures, such as *Ca. vicina* (Donovan *et al.*, 2006), *P. casei* (Russo *et al.*, 2006), and *Lucilia sericata* (Roe and Higley, 2015), are more likely to provide problems in PMI estimation using ADD following refrigeration as their continued development may skew the PMI estimation if refrigerator temperatures fluctuate too greatly. Walker *et al.* (2013), recommends that the temperatures within refrigerators must be evaluated and if significant variations are present appropriate measures must be taken in the analysis of insects taken from human remains. This is an area for concern though, as it requires death scene technicians and/or mortuary staff to ensure they are recording these temperatures and that the temperatures are not merely assumed to be that of the set temperature of the refrigerator. The findings of the present study agree with the suggestions of Higley and Haskell (2010) that there is a need to clearly understand the variations present in PMI estimations.

Variations do exist in mortuary refrigerators, as found in this study with temperatures ranging from -4.44^{o}C to 22.32^{o}C due to mechanical failures in the refrigerator. This failure in the refrigerator did increase the variability above and beyond that of a "normal" scenario but for the purposes of this study it presented a perfect "worst-case" scenario which could be used to test high levels of variation using Monte Carlo simulation. There is a need to understand what factors cause these variations or at least in ensuring that they are adequately recorded to aid in the accuracy of PMI estimations (Walker *et al.*, 2013). Lack of adequate death scene temperature information and data collection can also affect the estimation of the PMI (Hofer *et al.*, 2017). The results of the present study provide a good baseline of potential "worst-case" temperature variations using the mean and standard deviation to provide a range in the ADD estimation. While many authors have investigated the accuracy and precision of PMI estimation (Dabbs, 2010; Villet *et al.*, 2010), the accuracy of the data collected is important. Death scene investigators and mortuary officials can aid in collecting these data. Failing this there is a need to implement new measures such as data loggers on mortuary vehicles to monitor external temperatures and data loggers within body bags (when used) to monitor temperature conditions to which the body is exposed.

In conclusion, the refrigeration may halt insect development but the effect this has on subsequent insect development and estimation of the PMI using thermal summation methods needs to be carefully considered (Johl and Anderson, 1996; Myskowiak and Doums, 2002). The findings of the present study suggest that, while more research to increase the accuracy of PMI estimations is needed, better management procedures of those working with the dead and the data they collect may be just as important. This study did not look at the effect that the number of bodies or human remains had on the temperature of the refrigerator as this effect was not considered until after the data were being analysed. It is therefore suggested that future research observe the number of bodies present within the refrigerator and the effect this may have on temperature fluctuations. The period during which the refrigerator doors were open during movement of bodies into and out of the refrigerator was also unknown. Consideration also needs to be given to different insect species, presence of body bags, presence of maggot masses, stage of larvae when placed into the refrigerator, period within the refrigerator, and accuracy of refrigerator temperature gauges.

CHAPTER FOUR

Conclusion and Recommendations

The study of human bodies received at the Johannesburg Forensic Pathology Service identified the most common forensically important insect taxa colonising human bodies in various stages of decomposition. Bodies colonised by insects make up a relatively small proportion of the total number of bodies received at the Forensic Pathology Service. In the cases where insects are present there is usually a considerable lack of information from the death scene which means that often insufficient information is available for death investigators. When the bodies have undergone considerable decomposition before being recovered and sent for a medico-legal autopsy, one of the most important pieces of information required is the post-mortem interval. In these cases, insect colonisation can provide valuable information to aid in forensic investigations (Ambade *et al.*, 2011).

This research identified 15 insect taxa associated with human bodies. The following Diptera were found: Calliphoridae: *Calliphora vicina*, *Chrysomya albiceps*, *Chrysomya chloropyga*, *Chrysomya marginalis*, *Lucilia sericata*; Muscidae: *Musca domestica*; Sepsidae: *Nemopoda sp.*; Piophilidae: *Piophilia casei*; Sacrophagidae: *Sarcophaga cruentata*. In addition, Coleoptera: Cleridae, Dermestidae, Histeridae and Staphylinidae; Phthiraptera: *Pediculus humanus* var. *corpus* were found. Seasonal differences were noted with winter having the greatest species richness followed by summer, which agrees with that of Kelly (2006). This study found that temperature was the most important variable affecting the insect taxa present on the body. A combined effect of temperature and the stage of decomposition was found to be important in determining the number of taxa present in each case. These findings agree with those of previous researchers (Bharti and Singh, 2003; Williams and Villet, 2006; Anderson, 2010; Villet, 2011; Gilbert, 2014).

The time since colonisation by the insects sampled from these bodies is an important tool that provides information needed to estimate the post-mortem interval (PMI) (Amendt *et al.*, 2007). Accurate estimations of the PMI are only possible following appropriate collection techniques of both the insects and environmental data (Amendt *et al.*, 2007; Richards and Villet, 2009; Villet and Amendt, 2011). The collection of insects for PMI estimations should occur initially at the death scene and later at the autopsy (Amendt *et al.*, 2007; Byrd *et al.*, 2010), for comparative purposes. In many cases, insect evidence is only collected at the

autopsy (Johl and Anderson, 1996; Huntington *et al.*, 2007) or even after the autopsy (Thevan *et al.*, 2010). The results shown in this study suggest that there is a considerable margin of error following refrigeration which may potentially have an effect on the ability to determine an accurate PMI. With careful monitoring, however it may still be possible to correct for refrigeration depending on the insect species or taxa collected and their developmental thresholds.

4.1 Study Limitations and Recommendations for future research design

This study started with the intention of attending death scenes and collecting insects from the death scenes to be compared with those collected from the same bodies at the time of autopsy. This proved to be a difficult task because insufficient information was given by the police to the forensic officer who attended the scene, resulting in bodies being moved and transported before I had a chance to attend the scene. This meant that the initial collection of insects became impossible. This would have been valuable information and the findings would have provided a better understanding of the factors discussed in Chapter 3. If temperatures and insect collections had been possible at the death scene it would have enabled a more accurate estimation of the post-mortem interval which could have been compared with simulated estimations. This would allow for the determination of the accuracy of these estimations in addition to comparing if there were any discrepancies in the insects sampled at the scene compared to those sampled at the autopsy. Such discrepancies may result from disturbing the remains when the body is removed from the scene, loss of insects during transport and migration of insects off the body during refrigeration. Simulated studies using human analogues may provide a better method to assess the effects of removal of the body, refrigeration and the autopsy before insects are collected. This would avoid the problems encountered in the present study and it would ensure greater control of the variables, such as temperature and disturbance, which may impact PMI estimation and hence the time of death determination.

This study also collected the data from a mortuary refrigerator to simulate variations in PMI estimates. The effects of different refrigerator temperatures on ADD determination is one area where future researchers can expand the understanding of temporary cooling, larval survival and the effect on development after a period of cooling. The potential of Monte Carlo simulation in describing variations in estimates, as suggested by Higley and Haskell (2010),

provides a possible method for increasing the objectivity of PMI estimates. Body bags were not used at the time of this study but they have now become standard procedure in all cases. Determining the effects of temperature variations both within and outside of body bags would provide important information which may affect the development of different fly larvae. In addition to this the effect of the number of bodies present within the refrigerator would be an important variable to record as this may alter the temperature due to bacterial activity, artificially increasing the refrigerator temperature with an increasing number of bodies. The impact of this was not considered until the time of writing this dissertation.

Overall this study encountered major limitations because of the difficulty in controlling all the variables in a real-world scenario. It is therefore essential that future research continue the use of human analogues under controlled conditions or using human remains where possible. While the results of this study provide some valuable insight into the implications of PMI estimation in the real world, they do highlight the need for further controlled experiments to better understand the factors which affect PMI estimation.

4.2 Recommendations for forensic services

Scene attendance is important to ensure that all taxa that were present at the scene remain with the body. Some species may burrow into the soil or move to the surrounding area when disturbed. The temperature and environment of the scene is also important for understanding the conditions to which the body was subjected. This information is often not recorded and when it is, the information is general and not useful (e.g. temperature: “Warm”, Scene type: “Outside in plants”). This information is very important, especially to the entomologist who needs to understand the zoology and ecology of the area from which the body was removed. It is therefore recommended that forensic officers take photographs of the scene and make an attempt to observe insects around the body, unless an entomologist is present. The presence of experts at the death scene is also a major issue. Forensic experts, except police technicians, rarely attend the death scene even in the case of forensic pathologists. Valuable information is lost from this and it is suggested that law enforcement and forensic investigators integrate and coordinate better to improve the quality of service provided.

In South Africa forensic entomologists are rare, the profession does not formally exist, and academic experts typically fulfil the role. These experts are consulted by the police on a case-by-case basis with primary focus on “high-profile” cases (those in the media). This means that

many cases that would benefit from forensic entomological analyses never receive any. None of the cases reviewed in this study had any further analysis of the insect colonisers than what was performed in this study. Many of these cases had no time of death estimation at all. The employment of one forensic entomologist per major metropole would greatly benefit the forensic pathology services in South Africa.

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APPENDICES

6.1 Summary of taxa sampled

Order	**Family**	**Genus**	***Species and taxonomic authority***	**Notes**
Diptera	Calliphoridae	*Calliphora*	*vicina* Rob.-Desvoidy, 1830	Adventive species with limited data on it's distribution in South Africa (Williams and Villet, 2006)
Diptera	Calliphoridae	*Chrysomya*	*albiceps* Wiedemann, 1819	Common species throughout southern Africa (Zumpt, 1965)
Diptera	Calliphoridae	*Chrysomya*	*chloropyga* Wiedemann, 1818	Common species throughout southern Africa (Zumpt, 1965)
Diptera	Calliphoridae	*Chrysomya*	*marginalis* Wiedemann, 1830	Common species in Africa but poorly described (Zumpt, 1965)
Diptera	Calliphoridae	*Lucilia*	*sericata* Meigen, 1826	Diurnal species with a wide distribution throughout South Africa and globally (Zumpt, 1965; Picker *et al.*, 2004)
Diptera	Muscidae	*Musca*	*domestica* Linnaeus, 1761	Ubiquitous and cosmopolitan species (Picker *et al.*, 2004)
Diptera	Sepsidae	*Nemopoda*	Robineau-Desvoidy, 1830	Worldwide distribution with corpophagous larvae (Smith, 1986)
Diptera	Piophilidae	*Piophilia*	*casei* Linnaeus, 1761	Adventive species introduced into the southern hemisphere (Zumpt, 1965)

Diptera	Sarcophagidae	*Sacrophaga*	*cruentata* Meigen, 1826	Adventive species which is a well-known synanthropic fly (Zumpt, 1972)
Phthiraptera	Pediculidae	*Pediculus*	*humanus var. corpus* Linnaeus, 1758	Common species with a wide distribution, occuring only on the human body (Picker *et al.*, 2004)
Hymenoptera	Formicidae	-	Latreille, 1809	Actual species not identified. Opportunistic feeders (Smith, 1986)
Coleoptera	Cleridae	-	Latreille, 1802	Limited distribution, mostly found in the northern and eastern regions of South Africa. Predacious (Picker *et al.*, 2004)
Coleoptera	Dermestidae	*Dermestes*	*maculatus* De Geer, 1774	Widely distributed throughout South Africa except the south-western interior (Midgley, 2007)
Coleoptera	Histeridae	-	Gyllenhal, 1808	Widely distributed throughout South Africa, predacious, synathropic but modern taxonomic keys lacking (Villet, 2011)
Coleoptera	Staphylinidae	-	Lameere, 1900	Widespread in South Africa, predacious, but limited taxonomic research on carrion species (Villet, 2011)

6.2 Ethics Clearance Certificate

R14/49 Mr Lawrence Hill

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M170781

NAME: (Principal Investigator)	Mr Lawrence Hill
DEPARTMENT:	Forensic Medicine and Pathology School of Pathology
PROJECT TITLE:	Forensic Entomology and Post-mortem Interval Determination from a Sample taken at the Johannesburg Forensic Pathology Services
DATE CONSIDERED:	26/08/2011(Initial Approval 10/07/2017)
DECISION:	Approved unconditionally
CONDITIONS:	Renewal for 5 Years Valid for the Period 01 July 2017 - 31 July 2022 (Previously M110835)
SUPERVISOR:	Dr Guinevere Gordon
APPROVED BY:	Professor CB. Penny Co-Chairperson, HREC (Medical)
DATE OF APPROVAL:	15/09/2017

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 10004,10th floor, Senate House/3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand. I/We fully understand the conditions under which I am/we are authorised to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. in this case, the study was initially review July and will therefore be due in the month of July each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

6.3 Facility permission letter

Umnyango wezempilo no Kuthuthukiswa Komphakathi
Lefapha la Maphelo le Tshebeletso le Ntshetsopele ya Sechaba
Department of Health and Social Development
Departement van Gesondheid en Maatskaplike Ontwikkeling

Prof Jeanine Vellema
Chief Specialist & Head of Division
Forensic Pathology Service: Johannesburg
Tel: 011 -480 1050/00
Cell: 082 777 0737
Fax: 086 050 8413
E-mail: vellema@telkomsa.net

TO WHOM IT MAY CONCERN
RE: RESEARCH REQUEST BY MR. LAWRENCE HILL

Permission is hereby granted for Mr. Lawrence Hill to conduct his research as proposed. He is being granted access, pending ethics approval, to the autopsy suite to observe the autopsies as they are carried out as well as access to the scribe notes/ autopsy report with the understanding that the strictest confidentiality will be maintained and that all data will remain anonymous and unlinked.

Yours truly,

.....
Prof Jeanine Vellema
Professor, Chief Specialist & Head of Division
Forensic Pathology Service: Gauteng Southern Cluster

6.4 TurnItIn Originality Report

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