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ABSTRACT

Decomposition cases form a fraction of the medico-legal cases conducted at Salt River Mortuary (SRM). With prolonged time since death and increased decomposition, entomological evidence becomes increasingly important in the estimation of minimum post-mortem interval. Currently no standard protocol exists for the handling of entomological evidence by SRM personnel and there is a lack of information about the issues that may impact the handling of these death scenes and associated entomological evidence. Therefore, this study aimed to investigate the current scope of cases involving entomological evidence at SRM. This was achieved by performing a six-year retrospective review of medico-legal cases performed at SRM from 2015 to 2020, and interviewing SRM personnel to gather data regarding attended scenes, methods and processes used on the scenes, and issues they may have faced.

A total of 264 decomposition cases were examined at SRM in the six-year period, with 109 (41.3%) presenting with insect activity. Data about variables such as scene type, weather season, decomposition stage, burial or covering of remains, and open wounds were extracted from the case files. As expected, a greater proportion of cases presented with entomological evidence in the warmer summer and spring seasons compared to the cooler seasons, with no significant difference in the distribution between years ($p=0.62$). Insect activity was predominantly found in indoor cases, but this is not statistically significant ($p=0.50$). Most cases presenting with entomology activity were associated with early-stage decomposition. No association was observed between the presence of open wounds and insect activity.

The interviews provided data that could not be extracted in the reviews, due to personal experience being provided by personnel. The primary themes emerging from the interviews were related to the insufficient training on the handling of entomological evidence, poor availability of resources for the handling of the entomological evidence, and scene dependent variables that differ between scenes and impacts how a scene is handled.

This study identified areas that need improvement and provides a better understanding of entomological activity associated with decomposition cases. There is potential for the greater utility of forensic entomologists in medico-legal cases, and the implementation of a standardised entomological protocol along with proper training of personnel may improve medico-legal investigations of decomposition cases.

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ABBREVIATIONS

ABFE	American Board of Forensic Entomology
ADD/ADH	Accumulated degree days/hours
DNA	Deoxyribonucleic acid
EAFE	European Association of Forensic Entomology
ENT	Entomology evidence collection kit
FP	Forensic pathologist
FPO	Forensic pathology officer
FPS	Forensic Pathology Service
PMI	Post-mortem interval
SAECK	Sexual assault evidence collection kit
SAPS	South African Police Service
SRM	Salt River mortuary

CHAPTER 1: LITERATURE REVIEW

1.1. Introduction

Forensic entomology is the science that analyses the life stages and colonisation behaviour of insects and associated arthropods to assist in drawing up conclusions for legal cases, which may be either civil or criminal in context (Magni *et al.*, 2013). Criminal cases refer to the medico-legal cases involving living humans or human remains that have been infested by insects, or where a potential criminal act has occurred. The use of forensic entomology, and the knowledge of the relationship between insects (typically blow flies) and death, goes as far back as 13th century China (Benecke, 2001; Wang *et al.*, 2021). When it comes to medico-legal death investigations in contemporary society, the combined efforts of multiple disciplines play a crucial role. Where a forensic pathologist is unable to provide a conclusion in an investigation, such as the determination of the post-mortem interval (PMI), the forensic entomologist can step in to assist (Campobasso and Introna, 2001).

The implementation of forensic entomology in medico-legal cases has proven to be valuable, as it can result in accurate estimations of PMI_{min} (Catts and Goff, 1992; Amendt *et al.*, 2004; Pittner *et al.*, 2020), but the field is not limited. Through the years, the ongoing research and practice of forensic entomology has progressed, and its implementation has expanded with regards to how it can be used in medico-legal cases. Insects associated with medico-legal cases can be used to provide information with regards to geographical location of death (Byrne *et al.*, 1995; Haskell *et al.*, 1997), movement or storage of human remains (Haskell *et al.*, 1997; Matuszewski *et al.*, 2013), trauma sites on severely decomposed remains (Haskell *et al.*, 1997), drug detection (Chophi *et al.*, 2019) or finding human DNA to identify suspects in sexual assault cases (Chamoun *et al.*, 2020) or victims themselves (Durdle, 2019).

The expertise of forensic entomologists can assist forensic pathologists during medico-legal death investigations, especially when it comes to determining time of death or PMI. In most cases, the pathologist can use post-mortem changes to determine the PMI. However, this method is often limited to the first 72 to 96 hours after death, after which thanatological changes become less helpful (Goff, 2009). The knowledge regarding insects' or arthropods' life stages and their carrion colonisation behaviour is useful for estimating PMI beyond the first 72 hours (Amendt *et al.*, 2006; Pittner *et al.*, 2020).

As with any field of forensic science, proper evidence sampling techniques are vital for quality entomological analyses (Amendt *et al.*, 2006; Amendt *et al.*, 2011; Byrd and Sutton, 2020). These techniques are easy to adhere to when a forensic entomologist is present on a scene; however, this is not always the case due to lack of forensic entomologists (Campobasso and Introna, 2001; Amendt *et al.*, 2006; Amendt *et al.*, 2015; Lutz *et al.*, 2021). This means that when a forensic entomologist is called upon, they need to work with information and evidence collected by third parties, such as the forensic officers or pathologist who attends the scene. This creates several issues, since untrained or unprepared personnel may miss the oldest developmental stage of insects (Campobasso and Introna, 2001; Lutz *et al.*, 2021) or handle the samples incorrectly (Bugelli *et al.*, 2017; Archer *et al.*, 2018), leading to problems during the analyses. Furthermore, it is often that sampling of entomological evidence is only done when arthropods are seen to be associated with the body at the time of autopsies (Huntington *et al.*, 2007; Lutz *et al.*, 2021). This is a major limiting factor as the autopsy may only be conducted days after the body is discovered, therefore limiting the amount of valuable information which can be obtained.

There is a need to assess current practices with regards to the recovery and handling of entomological evidence during medico-legal death investigations, and establish standard protocols which personnel can follow in the absence of a forensic entomologist (Lutz *et al.*, 2021). Furthermore, regular training in forensic entomology practices for forensic pathologists and forensic pathology officers could assist in improving future medico-legal death investigations (Gaudry *et al.*, 2001; Lutz *et al.*, 2021).

1.2. Forensic entomology

1.2.1. History of forensic entomology

Forensic entomology is not a new science, however, the routine application of forensic entomology in a medico-legal context has only been practiced since the second half of the 20th century, particularly by developed nations in North America, parts of Europe and Asia (Benecke, 2001), and Australia (Archer and Wallman, 2016). In many locations, such as South Africa, forensic entomology is not yet considered routine practice and is still in its infancy. Provided below is a summary of the history of forensic entomology. For further reading, Benecke (2001) provides a thorough review of the global history of forensic entomology, while reviews of the history of forensic entomology practice and research in South Africa have been presented by Williams and Villet (2006) and Tembe and Mukaratirwa (2020).

The history of forensic entomology can be traced back to the first documented use of insects during a murder investigation in 13th century China (Benecke, 2001; Wang *et al.*, 2021). The investigator appointed to the crime asked suspects to lay down their sickles (the tool determined to be the murder weapon). Only one attracted flies – due to traces of blood that the murderer was unable to clean off his sickle (Giles, 1924). This concept was later re-enforced by studies showing certain blow flies prefer the blood of a corpse over decomposing tissue (Benecke, 2001). The understanding of the relationship between decomposing flesh and insects also went beyond medical and legal experts. Many artists have illustrated the feeding of maggots on corpses, with accurate depictions of the feeding patterns of the insects – leading to early skeletonisation of the skull and loss of internal organs (Benecke, 2001). The poem "Une Charogne" ("A Carcass") by renowned French poet Charles Baudelaire (1821-1867) is known to depict thorough observations on the decomposition of human remains, and he further describes the sounds of maggot masses feeding (Baudelaire, 1998).

Moving into western society, it was in 1668 when Francesco Redi demonstrated that maggots do not spontaneously appear on decaying flesh. Instead, flies were being attracted to chemicals released by the decomposing remains and would lay their eggs, which hatched into maggot larvae and used the remains as a feeding ground (Lambiase and Gemmellaro, 2015). In the early 20th century, it was entomologist Giuseppe Müller (1880–1967) that was the first to recover and preserve insect evidence found in a suspected case of suicide by hanging (Lambiase and Gemmellaro, 2015). Research does not indicate why he did this, as the recovered evidence was not used to draw conclusion to the case. Müller was more interested in systematic and phylogenetic questions, and the specimens he collected are still on display at the Museum of Trieste. At the time, no other entomologist was performing recovery and preservation of forensic insect evidence.

It was in 1850 near Paris, France, when the science of forensic entomology was used to assist in estimating the date at which human remains were placed into a house wall (Bergeret 1855, as cited in Benecke (2001)). Insect evidence found with the body was used to absolve the current homeowners, as they would not have been present at the time that the remains were hidden in the wall. However, the forensic examiner, Dr L. Bergeret, did not have a full understanding on the development cycle of the insects, as he made errors such as stating female flies will only lay eggs during summer and pupae would only hatch the following summer (Bergeret 1855, as cited in Benecke (2001)). Bergeret only used forensic entomology as a tool to aid in his investigation, as he noted knowledge about insect succession on corpses was lacking in his time, and his primary interest in the case was the mummification of the corpse (Benecke, 2001).

With the efforts of Yovanovich and Mégnin and their research on insect succession, the field of forensic entomology and its data was boosted (Amendt *et al.*, 2004). Mégnin was the first to present data on various stages of decomposition, along with data on the possible insect and arthropod species that may be found during each stage (Mégnin, 1894), which was later re-defined by Goff (1993). However, it was the work of scientists like Leclercq (1983) and Nuorteva (1974) that saw forensic entomology being used to assist in estimating the PMI in medico-legal death investigations, thus introducing forensic entomology as it is used today into the 20th century. Since then, further research has brought forensic entomology into the 21st century and made it a highly accepted forensic tool during medico-legal investigations (Goff, 1991; Goff and Flynn, 1991; Catts and Goff, 1992; Campobasso and Introna, 2001).

Research and progress in forensic entomology has been ongoing in the northern hemisphere but has been slow in the southern hemisphere despite its vast diversity of forensically important insects. Over the last century, entomology research in South Africa was mostly on insect identification, development, and succession on animal corpses (Ellison, 1990; Williams and Villet, 2006). Soon experts realised the information about insect succession on animal corpses could be applied to human remains and research expanded into the insects that frequent human remains and how these insects could aid in a forensic investigation (Louw and van der Linde, 1993). Further research by entomologist T.C. van der Linde went into topics such as development rates of maggots (Leipoldt and van der Linde, 1993 as cited in Williams and Villet (2006)), maggot anatomy (Brink, van der Linde, Basson and van Wyk, 2005 as cited in Williams and Villet (2006)), and insect succession on clothed or wrapped corpses (Cronje, Louw and van der Linde, 1995 as cited in Williams and Villet (2006)). Experts further began research into the identification of insects and arthropods using mDNA (Harvey *et al.*, 2003).

The first forensic case in South Africa that made use of forensic entomology to reach a conviction was in 2000. This was the case of Albert du Preez Myburgh, who was being charged with indecent assault and murder, and the entomological evidence had been provided by Mervyn W. Mansell (Williams and Villet, 2006). Mansell had previously been contacted by the South African Police Service (SAPS) to work on the Moses Sithole case in 1995, and this association led to Mansell's involvement in many high profile cases in the early 2000s (Williams and Villet, 2006).

The field of forensic entomology in South Africa is still expanding, although most research is being undertaken without a forensic context (Tembe and Mukaratirwa, 2020). However, the research that has been conducted still has many useful results that can be translated to a forensic context, or serve as foundations for further research with a forensic context (Tembe and Mukaratirwa, 2020) that may be useful with regards to medico-legal investigations.

1.3. Medico-legal death investigations

1.3.1. Post-mortem changes

After death, there are several changes in the body. These can either be early post-mortem changes (skin pallor, relaxation of skeletal muscles, dark bands in the eye known as 'tache noir') or late post-mortem changes (rigor mortis, livor mortis, and algor mortis). The changes occur over a period, starting as early as 2 hours after death to 96 hours after death. Internal or external factors, associated with the remains or environment, can play a role in this period by either delaying or accelerating it. The post-mortem changes are a result of the autolysis process due to the breakdown of cells and release of enzymes, as well as bacterial and fungal activity (Clark *et al.*, 1997; Amendt *et al.*, 2004). Autolysis is just one aspect of the decomposition of a body, which is a continuous process that can be broken down into five stages: fresh (autolysis begins here), bloated, decay, post-decay, and skeletonisation (Goff, 2009).

During the stages of decomposition, insects are attracted to the remains and may aid in decomposition. Different insect species will be attracted to human remains at different points in the decomposition stage depending on temperature (Smith, 1986), as it fits their ecological need for food and breeding opportunities.

1.3.2. Insect colonisation

Insects, especially Diptera (blow flies), will be attracted to the chemical signals released by decomposing human remains within minutes if the temperature is optimal for their activity (Haskell *et al.*, 1997; Tabor *et al.*, 2005). The attracted insects can be divided into four categories: necrophagous species; predators and parasites of necrophagous species; omnivorous species; and adventive or opportunistic species (Goff, 1993).

Necrophagous species will use decomposing tissue as a food source, while the predators and parasites will be attracted to the necrophagous species colonising human remains. Omnivorous species will be attracted to both decomposing tissue and other insects as a food source, while the adventive species use the remains as a potential habitat (Goff, 1993; Goff, 2009). Another category that is not typically recognised is incidental species. These species have no direct relationship with carrion or insects attracted to carrion and their presence on or near a body is purely coincidental. Nevertheless, such arthropods can be used to infer information regarding movement of a body from one location to another, and should therefore not be ignored (Goff, 2009).

While the first two categories of species are most used in the application of forensic entomology (Boehme *et al.*, 2012; Szelecz *et al.*, 2018), each category can be of evidential value depending on the case and decomposition stage of the human remains (Goff, 2009). It is therefore important that sampling on a scene is done correctly, to ensure all species are represented during analysis and allow for highly accurate PMI estimations.

1.3.3. Temperature and PMI estimations

Temperature plays an important role in the calculation of PMI. Firstly, temperature will influence the colonisation of human remains by insects (Smith, 1986). During warmer seasons, certain species may colonise remains at a higher rate because they prefer the warmer temperatures, while a species that prefers cooler temperatures may colonise remains in fewer numbers or be entirely absent (Williams and Villet, 2019). Secondly, the temperature and fluctuation thereof may influence the rate of development of insects. The rate of development of an insect through its' various life stages may be absent or slow when temperatures are low, increase as temperatures increase then begin to slow down as temperatures get higher (Higley and Haskell, 2010). If temperatures get too high and remain that way, it may prove deadly for the insects (Higley and Haskell, 2010). Furthermore, the optimum temperature for one species to development may differ from another species, therefore both temperature and identification of the insect species are important when estimating PMI intervals.

When determining PMI, the forensic entomologist will first identify any forensically important insect species that were present with the remains. The next step would be determining the rate of development of the various life stages of the insects, which relies on temperature (Smith, 1986; Amendt *et al.*, 2006). This may require the temperature of the larval masses, of the remains (if the insects were on the body i.e., larval stages) or the ambient temperature of the environment (if insects were found next to or near the remains i.e., adult stages) (Amendt *et al.*, 2006). It is important to record these various temperatures at a death scene, as it may not be immediately obvious which insect species are present, and thus which temperatures will be of importance for PMI estimation (Amendt *et al.*, 2006). In some cases, the investigator may leave data loggers on the scene to record temperatures over a certain period, or contact the closest weather station to gather records about the temperatures of the area in which the remains were found (Charabidze and Hedouin, 2019).

While there are many methods to estimate PMI using insects, a well-researched method is accumulated degree days/hours (ADD/ADH) (Amendt *et al.*, 2006). This method takes the summation of temperatures experienced by a specimen over the period of growth to determine the age. The ADD/ADH between insect species will differ, as the temperature required for each developmental stage to progress may differ between different species (Amendt *et al.*, 2006). While this method is very useful in determining PMI, it must be used with caution. There has been a report on cases where the ADD/ADH produced a result that was different to the actual PMI determined through security camera footage (Guo *et al.*, 2023). The remains in this study were found under thick quilts, which affected the temperature of the remains, thus resulting in an PMI calculation that was different to the actual PMI. Studies like this indicate the need to take thorough notes regarding the remains and conditions thereof as well as the surrounding environment, before leaving a scene.

1.4. Standardised practices

As with all the sciences and especially forensic sciences, the aspect of quality assurance remains at the forefront (Randall *et al.*, 1998; Amendt *et al.*, 2006). When a standardised approach exists for the recovery and handling of evidence, one not only ensures that contamination or destruction is prevented, but that the chain of custody is maintained. Thus, a standardised approach is necessary for forensic entomological evidence. Furthermore, forensic entomological investigation involves the handling and rearing of living specimens which should be conducted in an ethical manner. Standard protocols are vital in a laboratory setting, however, research has shown that making use of standard protocols from the onset of evidence recovery, whether at a scene or the mortuary, may ensure no errors occur downstream (Archer and Wallman, 2016). One standard protocol may not fit all the settings of a medico-legal investigation, but multiple protocols can be developed to assist at each step in the investigation (Gomes and Von Zuben, 2006; Archer and Wallman, 2016). The implementation of standard protocols, at any forensic setting, will further ensure that the correct procedures are being followed, even in the absence of the relevant expert (Gomes and Von Zuben, 2006).

The issue with the recovery and handling of entomological evidence on scene is that a forensic entomologist may not always be present (Campobasso and Introna, 2001; Byrd and Sutton, 2020; Lutz *et al.*, 2021). With no standardised procedure in place, this creates the issue of personnel on scene not understanding the importance of collecting entomological evidence, missing certain life stages or even insects they may think not forensically important (Lutz *et al.*, 2021), or handling them incorrectly (Bugelli *et al.*, 2017; Archer *et al.*, 2018). With the immense amount of information pertaining to forensic entomology, it is crucial that anyone involved in medico-legal death investigations understands the importance of entomological evidence and the need for proper recovery and handling of such evidence. A thorough death scene investigation that presents with insect activity, will involve the combined efforts of a forensic entomologist and forensic pathologist considering all the evidence that could lead to a conclusion on aspects such as time of death and the cause of death.

Over the years, many standard procedures for different scene environments or at autopsies have been developed to ensure correct handling and recovery of the entomological evidence (Campobasso and Introna, 2001). Currently, the best practices suggest the following sequence for entomological investigation on a crime scene: document any visual observations, collect climate and temperature data, collect specimens from the body before its removal, and collect specimens that were under the body (after removal), near the body and from the surrounding environment (Amendt *et al.*, 2006; Amendt *et al.*, 2015). The recovery and handling of entomological evidence can be a major process, and even more so should insect activity be quite diverse and overwhelming. Furthermore, entomological evidence may not be the only evidence found on a scene, and should personnel not have sufficient training for the recovery thereof (Amendt *et al.*, 2006; Gomes and Von Zuben, 2006), the overall handling of the death scene could be delayed. Overall, standard protocols assist with procedures that concern evidence as well as ensuring scenes are handled efficiently.

1.4.1. Standard practices and forensic entomology in South Africa

In the forensic setting of South Africa, unique circumstances experienced on different local scenes may emphasise the need for standardised approaches to recover entomological evidence. Currently, no standard protocols for entomological evidence are in use by the Forensic Pathology Service (FPS), and most entomologists who are asked for expert opinions are academics. Due to this fact, entomologists may not be called to a scene unless they have a history with assisting in medico-legal investigations (Williams and Villet, 2006). Having no entomologist on the scene means any personnel such as forensic pathologists (FPs) and forensic pathology officers (FPOs) may not understand the significance of entomological evidence or the correct procedures for recovering the evidence, especially if they lack the necessary training. Implementing standard protocols will not only ensure personnel know what to do but may help highlight what training may be required for personnel. The training could be provided by experts in the field of entomology and done intermittently to ensure personnel stay informed about current practices (Archer and Wallman, 2016). Other issues in the South African forensic setting that highlights the need for standard protocols are hostile environments that may limit the time available on scenes, or the lack of resources preventing recovery or handling of evidence. Development of standard protocols may assist in fixing issues of poor resource availability by ensuring personnel know what resources are needed for entomological evidence. Furthermore, development of a standard protocol for the forensic setting of South Africa will take into consideration any issues or challenges faced by personnel on different scenes. Personnel on scenes can thus ensure any entomological evidence associated with human remains will be recovered and handled efficiently. A standardised protocol will also indicate what to do when the remains arrive at the mortuary, especially when sampling could not be done at scene and is now required during autopsy (Lutz *et al.*, 2021).

1.5. Conclusion

Forensic entomology has been developing over many years. Considerable research has gone into the life cycles of various forensically important insects and how they may colonise remains as a method to estimate PMI, while other fields have joined with forensic entomology to use insects to infer information such as possible trauma sites, geographical location of the victim, assisting in identification of suspects or victims, and so forth. However, for these conclusions to be made, one must first ensure the proper recovery and handling of the entomological evidence. This is where standardised protocols play a crucial role.

By performing a retrospective review of cases presenting with entomological evidence and interviewing personnel that attend these scenes, this study will collect data on the unique environment of death scenes associated with insects and the challenges that come with them. This will aid in the development of any standardised protocol, while further highlighting other areas of forensic entomology in South Africa that requires improvements, such as the training of personnel to ensure they remain knowledgeable about current practices within the field of entomology. This will ensure that even in the absence of the forensic entomologist on a scene, personnel will have the necessary information and tools to ensure proper recovery and handling of evidence that could allow for thorough medico-legal death investigations.

CHAPTER 2: RESEARCH MANUSCRIPT

2.1. Introduction

The use of insects and arthropods to assist investigations, otherwise known as forensic entomology, is a practice that is becoming increasing utilised in medico-legal investigations around the world (Catts and Goff, 1992; Magni *et al.*, 2013). Commonly, forensic entomological practices are applied to determine the minimum post-mortem interval (PMI) (Campobasso and Introna, 2001) when examination of the corpse alone is not reliable (*i.e*., after approximately 72 hours when signs such as livor mortis and rigor mortis are absent). Much research has gone into the improvement of current PMI estimation methods that make use of insect evidence, such as identifying forensically important species and understanding their life cycles, however, other applications have also been recognised. Some of these applications include using insects' stomach contents to analyse drugs in the absence of viable human tissue (Catts and Goff, 1992; Chophi *et al.*, 2019), providing evidence in the form of species uncommon to the death scene area to suggest relocation of the decedent (Benecke, 2005; Matuszewski *et al.*, 2013), and even linking suspects to sexual assault cases (Chamoun *et al.*, 2020).

For entomology evidence to be reliable and useful, the correct handling and recovery thereof is of utmost importance during legal investigations (Amendt *et al.*, 2006; Byrd and Sutton, 2020). As certain applications of forensic entomology require identification of species, the best technique will be to start the evidence recovery at the scene to avoid missing out on any forensically important species or life cycles (Lutz *et al.*, 2021). Any recovered insects and arthropods will further need to be handled correctly to ensure no issues with any analysis that may be required (Archer *et al.*, 2018). Beside the proper recovery and handling of insect evidence, analysis of the evidence may require information about the scene and remains that could have affected the behaviour and presence of any species recovered. This information includes variables such as season and weather (Williams and Villet, 2019), decomposition stage (Tembe and Mukaratirwa, 2021), burial or covering of the remains (Kelly *et al.*, 2011; Bhadra *et al.*, 2014), as well as any trauma in the form of open wounds (Charabidze *et al.*, 2015). Should any of this information be missed, analysis of the entomology evidence may prove unreliable or even impossible.

Salt River mortuary (SRM) is one of 16 mortuaries in the Western Cape that falls under the administration of the Forensic Pathology Service (FPS) of South Africa and is one of two facilities with the M6 facility grade. This grade indicates that SRM conducts over 3000 post-mortems per year (Forensic Pathology Service, 2014), however, in recent years this number has exceeded 4000 cases per annum (Davies *et al.*, 2023). SRM provides forensic services to the West Metropole of the City of Cape Town, which encompasses multiple districts with over 1.9 million residents (Davies *et al.*, 2023). FPS and SRM are mandated by South African law to investigate all deaths suspected of being unnatural, which requires scene attendance and the collection of decedents and evidence associated with the remains. Forensic pathology officers (FPO's), and sometimes pathologists, will attend death scenes to recover and transport decedents, as well as document any information about the scene. Pathologists and FPO's are not the only professionals that attend death scenes, as the South African Police Service (SAPS) will also be present, as well as any relevant emergency service called onto the scene. Currently, entomology falls mainly under the purview of SAPS, and they will collect entomological evidence and consult entomologists (Williams and Villet, 2006) to assist with reconstruction of the death and surrounding circumstances. However, as entomological evidence can assist with PMI estimation, pathologists are allowed to collect entomological evidence or call in an entomologist for consultation (South Africa, 2018). Unfortunately, there is a shortage of forensic entomologists, as most are academic and only work as consultants. As no current standardised procedure exists for FPO's to follow, any scene involving insect evidence may be subjected to improper methods of recovery and handling of the insect evidence, and the aforementioned information required for entomological analysis may be missed or overlooked.

While entomological cases are not uncommon in the West Metropole of Cape Town, they might come with their own unique challenges, as several variables can play a role in the presence of insects and their behaviour at death scenes (Kelly *et al.*, 2009; Parry *et al.*, 2016; Tembe and Mukaratirwa, 2021). There has been no major review of medico-legal cases that have involved insects or arthropods previously handled by SRM. A review of this kind would provide a better understanding of these cases and their typical settings, the entomology evidence that may be present, and could highlight any issues or challenges that may be faced by personnel that attend the scenes. It is important to note challenges or issues that personnel face when attending scenes. Limited time spent on scene might indicate that certain processes on the scene must be sped up or completely ignored to ensure collection of the decedent or decedents, and in the case of entomological evidence, might mean that recovery of specimens can only be done at the mortuary. Therefore, it is important to gather as much information regarding entomological cases, including challenges associated with scene handling and collection of evidence.

The aim of this study was to investigate the current scope of decomposition cases and associated entomological evidence with emphasis on the SRM and the West Metropole of the City of Cape Town. Furthermore, this study aimed to gather data on current processes for the recovery, handling, documentation, and transportation of entomological evidence by Forensic Pathology Services. The objectives were to:

- Perform a retrospective review of cases with entomological evidence admitted to SRM from 2015 – 2020.
- Conduct semi-structured interviews with SRM personnel to ascertain current processes and perceptions for the handling and collection of entomological evidence.

2.2. Methods and materials

2.2.1. Study design

This study was conducted with a mixed methods approach using a parallel embedded design consisting of two phases. First, quantitative data were collected through a retrospective review of medico-legal cases conducted at SRM. In parallel with this, interviews were conducted with pathologists at SRM. The retrospective phase provided data about scenes and circumstances that may present with entomological evidence, while interviews provided more personal experience on scenes involving entomological evidence as well as issues or challenges that may be faced when dealing with such cases. When put together, the data form a bigger picture of the overall scope of current practices and scope for utility of forensic entomology. This may assist in developing a standard protocol that emphasises entomological evidence while still considering issues or challenges that may arise and would aid FPS personnel on any scene type. Each phase of the study is described further below.

2.2.1.1. Phase 1 – Retrospective review

A retrospective review of autopsies conducted at SRM between the period of 1 January 2015 to 31 December 2020 was conducted to collect information regarding medico-legal death investigations at SRM that were associated with decomposing remains and insect activity. This period was the most reliable as it provided a large number of complete case files to ensure sufficient data for the retrospective review. Relevant cases were identified using the Office Autopsy Database (OAD) (HREC REF: R036/2014). The OAD can be searched using terms like "decomposition", but not "entomological evidence" or "insect activity". Thus, as insect activity may be more prevalent in decomposition cases (Amendt *et al.*, 2004), the database was searched for decomposition cases within the 6-year review period. Cases were included if there were external signs of decomposition, regardless of insect activity, but excluded if it was referred to SRM from a hospital or funeral home, or the cases only presented with internal decomposition during post-mortem examination. External signs used to identify and confirm a decomposition case included skin changes (marbling, green discolouration, slippage) bloating of the abdomen and other body parts as well as the presence of purge fluid in the nose or mouth. Severe cases of decomposition would include early mummification, severe decomposition of wet tissue and/or adipocere formation, and loss of tissue resulting in exposure of bones (Galloway, 1997). Autopsy case files, which contain an autopsy pathology report, Lab 27, and FPS002 forms, were reviewed for contextual information regarding the scene (Table 1). This contextual information included variables such as date of death, state of decomposition, presences of wounds, concealment of remains, and presence of insects. As the pathology reports include details about remains, this was used to determine the signs of decomposition and classify each case as early, advanced or skeletonised (Galloway, 1997). Where autopsy reports were missing or provided insufficient information to confirm stage of decomposition, these were recorded as "unconfirmed".

Table 1: Relevant documents and data used.

Document in case file	Relevant data on the document
Contemporaneous Note (Lab 27) or Death Scene Script	Information regarding the death scene, contemporaneous notes from pathologist recorded during PM
FPS002 form (scene script)	FPO's complete this form on scene with case details, timelines, any observations made on scene that are pertinent to the case
Pathology reports	Information on decomposition, insect activity, trauma

2.2.1.2. Phase 2 – Interviews

The second phase of the study consisted of semi-structured interviews with forensic pathologists at SRM. The purpose of the interviews was to gather more information regarding the handling of cases involving entomological evidence and any data that could not be obtained from the reviewed files alone. Furthermore, the data gathered in this phase included feedback from the personnel who deal with these cases and could provide insight into possible improvements that could be made to processes used on scenes.

The personnel at SRM were asked to engage in online interviews (in keeping with COVID-19 and ethical regulations) that lasted a maximum of 25 minutes and were recorded if permission was granted. The interview process was managed with two questionnaires (Appendix A). The first questionnaire consisted of open- and close-ended questions pertaining to scene and autopsy details that are recorded, as well as details regarding evidence collection. The second questionnaire consisted of more open-ended questions that allowed participants to provide insight into challenges, issues, or risks they might have encountered with cases associated with decomposition and insects, as well as potential areas that could be improved. These questionnaires were completed with the participant during the interview sessions, and no personal information that could link a participant with a particular answer was recorded.

2.2.2. Data analysis

Quantitative data collected through the retrospective review was analysed descriptively. The collected data were recorded in a Microsoft Excel® spreadsheet (Microsoft, Washington, USA), which was also used to perform descriptive statistical analysis and create visual representation in the form of graphs. Pearson's chi-squared test was used to assess differences in relative distribution of variables and assess association between categorical variables. Statistical analysis was conducted using IBM SPSS Statistics 28 (IBM Corp, Armonk, New York), at the 95% confidence level.

Data obtained from the questionnaires were analysed qualitatively. The completed questionnaires from the interview phase were uploaded to Nvivo® software (version Release 1.0). If recording of the interviews was consented to by the participant, the recordings were reviewed in junction with the questionnaires to ensure all answers were recorded correctly and completely. Using Nvivo® and a general analysis approach (Braun and Clarke, 2006), an initial review of the data was performed, and multiple codes were generated that would be combined to form themes. Further review of the data allowed for newer codes to emerge or for codes to be combined into one theme if possible. Visual representation of the themes and subthemes was created using the Nvivo® software (version Release 1.0).

2.2.3. Data management and ethics

2.2.3.1. Privacy

All participation by interview participants in the study was voluntary. All data collected through the retrospective review or interviews were anonymised. This ensured that the data could not be linked to any specific forensic case or participant. Furthermore, no personal details of participants or decedents were collected to ensure confidentiality.

2.2.3.2. Ethical approval

This study was approved by the University of Cape Town (UCT), Human Research Ethics Committee (HREC REF: 249/2021 and HREC REF: 528/2021) (Appendix B and C). FPS approval to access the Office Autopsy Database (OAD) (HREC REF: R036/2014) was granted by the Head of Division of Forensic Medicine and Toxicology at UCT. As per the ethical requirements, the target participant group were notified of the study and provided with background information for the study. All participants provided informed consent before participating in the interviews (Appendix D).

2.3. Results

2.3.1. Retrospective phase

The OAD was reviewed for cases that fit the inclusion criteria during the period of 1 January 2015 to 31 December 2020. The mean number of cases assigned to SRM for the review period was 3830 per year, with the year 2020 having the lowest number of assigned cases (3495 cases) and the year 2019 having the highest number of cases assigned (4205 cases). The total number of cases for the six-year review period was 22982, of which 264 (1.1%) fit the criteria set out for this study. Files or digital copies of case files were retrieved, and relevant data were documented.

Decedents ranged in age from neonates to one decedent over 90 years of age, with most decedents belonging to the age group 31 to 40 years (Table 2). The mean age of decedents was 38.6 years (±22.1 years). Decedents in the various age groups were mostly male, except for the 0 to 10 year group which saw more female decedents. Across the remaining age groups, females were more evenly distributed. A chi-square test showed a significant association between age and sex ($p<0.001$). The alleged manner of death for the reviewed cases included accident (5.3%), homicide (11.4%), natural (31.1%) and suicide (6.4%). 121 cases were undetermined or still under investigation at the time of data collection.

Table 2: Age and sex of decedents in decomposition cases for the years 2015 to 2020.

Age range	Total	Sex	
		Male	Female
0 to 10 years	37	17	20
11 to 20 years	8	5	3
21 to 30 years	34	24	10
31 to 40 years	56	51	5
41 to 50 years	43	39	4
51 to 60 years	31	23	8
61 to 70 years	20	13	7
71 to 80 years	12	7	5
81 to 90 years	5	3	2
91 to 100 years	1	1	0
Unknown	17	-	-
TOTAL	**264**	**183**	**64**

2.3.1.1. Decomposition cases, seasonal distribution, and insect activity

For the period of 2015 to 2020, the seasonal distribution of decomposition cases (Figure 1) showed most of the cases occurred during the warmer summer (n=79/264, 29.9%) and spring (n=78/264, 29.5%) seasons, while they occurred less during the cooler winter season (n=51/264, 19.3%) and autumn (n=58/264, 21.9%) seasons. No significant difference was observed in the distribution of cases between seasons each year (p=0.62) (Figure 1). However, within 2020, the number of cases in each season were almost evenly distributed.

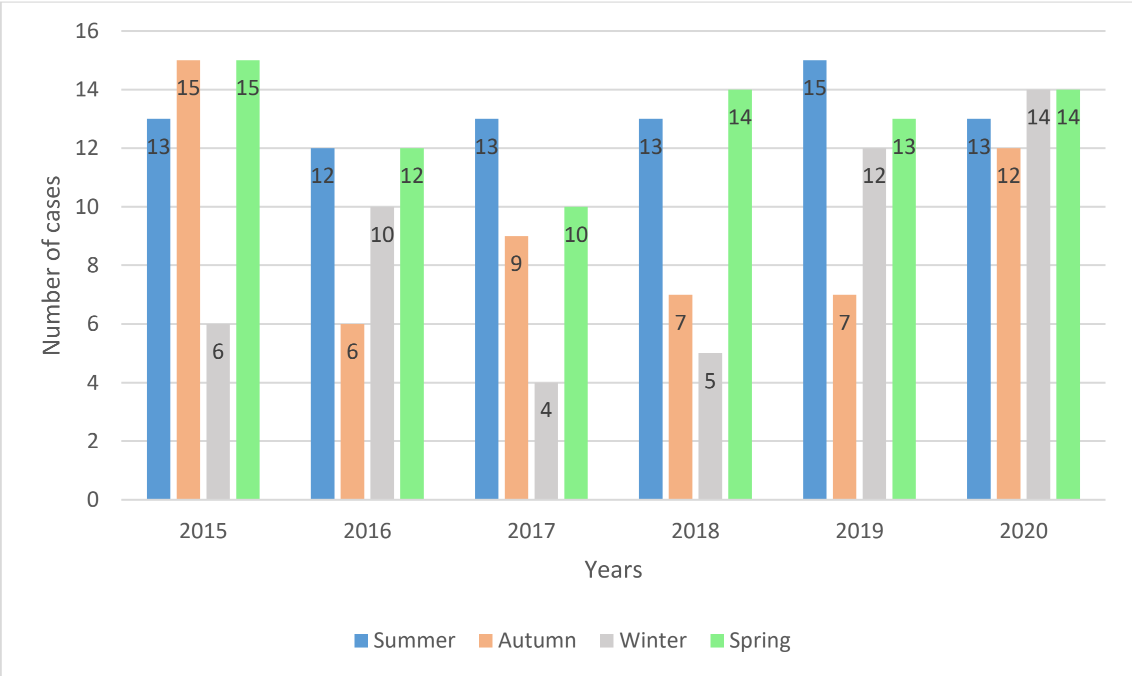

Figure 1: Seasonal distribution of decomposition cases for each year from 2015 to 2020.

The state of decomposition varied between cases (Figure 2). Most cases were in the early stage of decomposition (n=165, 62.5%) with signs of decomposition including discolouration, bloating, skin slippage, and/or marbling. Following this, advanced decomposition was observed in 55 (20.8%) cases, with signs including early mummification, severe decomposition of wet tissue and/or adipocere formation. Cases in the skeletonised stage of decomposition (n=6, 2.3%) included signs such as severe tissue loss resulting in major bone exposure or remains that were nearly complete skeletons but still had soft tissue due to clothes/shoes preserving certain areas.

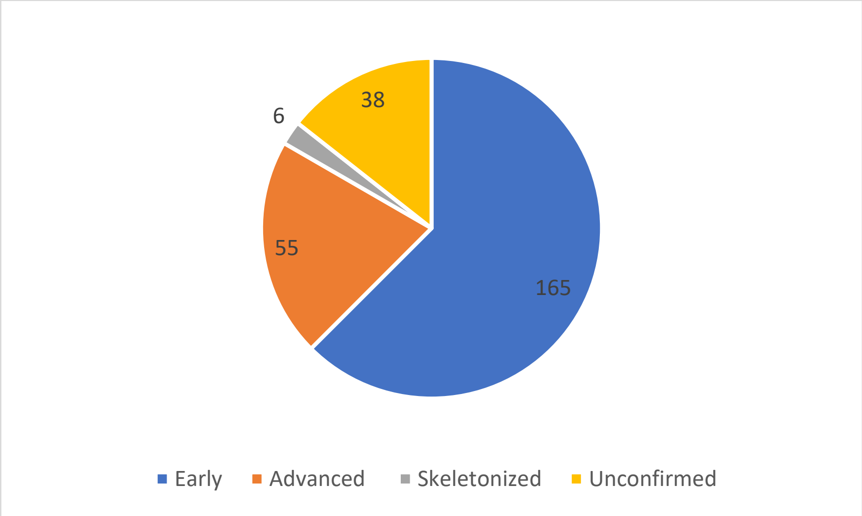

Figure 2: Breakdown of stages of decomposition.

2.3.1.2. Type of scene and geographic locations

Figure 3 shows the types of scene settings that were attended with regards to decomposing remains. Scenes were further defined as indoor (remains found inside a structure; n=135/264, 51.1%) or outdoor (remains found outside structure or in the open; n=129/264, 48.9%). Indoor scenes were predominated in the setting of formal housing (n=90/135, 66.7%) with fewer cases occurring in informal housing (n=37/135, 27.4%). The remaining eight cases were bodies retrieved from abandoned buildings (n=3), inside a vehicle (n=1), in a building on a sport field (n=1), a place of worship (n=1), a dormitory room (n=1), and a shopping mall restroom (n=1). Outdoor scenes were predominated by farm/open land settings (n=50/129, 38.8%) followed by areas of water and/or the beach (n=34/129, 26.4%).

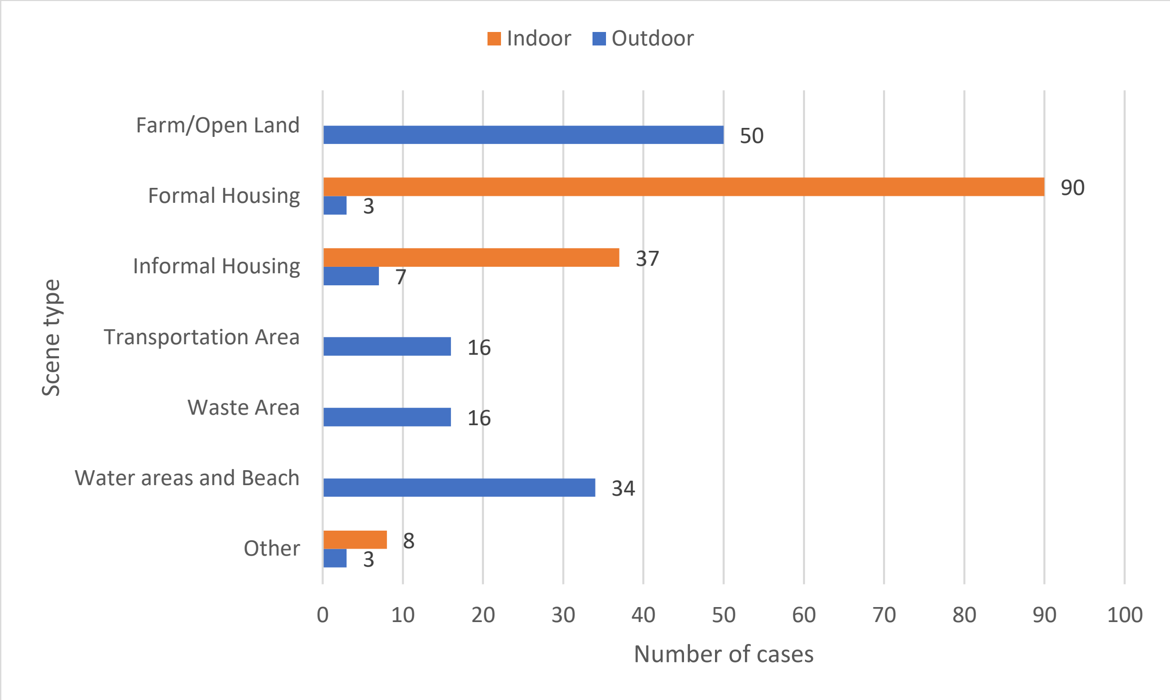

Figure 3: Types of scenes associated with decomposition cases.

'Transportation Area' includes roads, highways, railways, or parking lots. 'Waste Area' includes dumpsites, waste bins, or waste removal vehicles. 'Water areas and Beach' includes ocean, beach, rivers, lakes, canals, dams, streams. 'Other' includes sewage drains, abandoned buildings, miscellaneous buildings, and a vehicle.

Scene accessibility cannot be established through a retrospective review alone, especially since many exact locations could not be confirmed due to missing files, however, four case files provided enough information to establish those scenes as 'difficult to access'. The reasoning for this is that attending SRM personnel had to park vehicles in a suitable location (parking area or nearest road) before either hiking up a mountain to the scene (n=2) or manoeuvring around the rocky beach/shallows (n=2) to access the remains.

2.3.1.3. Insect Activity in decomposition cases

The number of decomposition cases and presence of insect activity is shown in Figure 4. In 38 cases (14.4%), there was either insufficient information available, or the case files were unavailable for review, thus the presence of insect activity in these cases could not be confirmed (labelled as 'unconfirmed' in graphs). Furthermore, case information rarely reflects what exact species are present, as insect activity is usually recorded by indicating the presence of flies, maggots, or other noticeable insects such as beetles. Insect activity was recorded in 109 of the decomposition cases (41.3%).

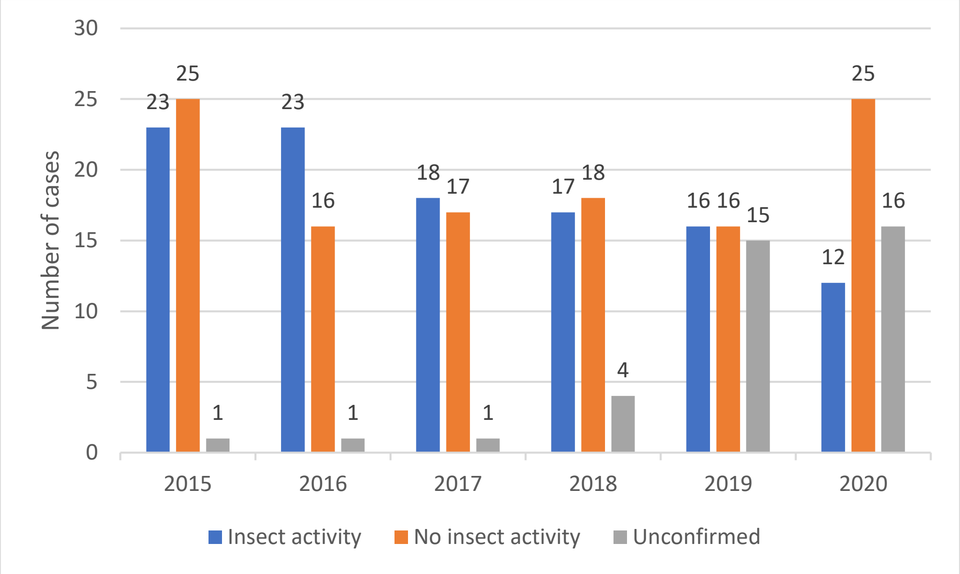

Figure 4: Insect activity in decomposition cases for the period 2015 to 2020.

For the cases associated with insect activity, data show there are fluctuations in case numbers between the different seasons within a given year (Figure 5). Overall, a significant difference was noted in the proportion of cases with entomological activity and season (p=0.02). This was predominantly due to a greater proportion of decomposition cases in summer having insect activity (n=41/79; 51.9%) and a low proportion of decomposition cases in winter with entomological evidence (n=10/51; 19.6%). No significant difference in the distribution of cases with entomological evidence per season was observed between the different years (p=0.71).

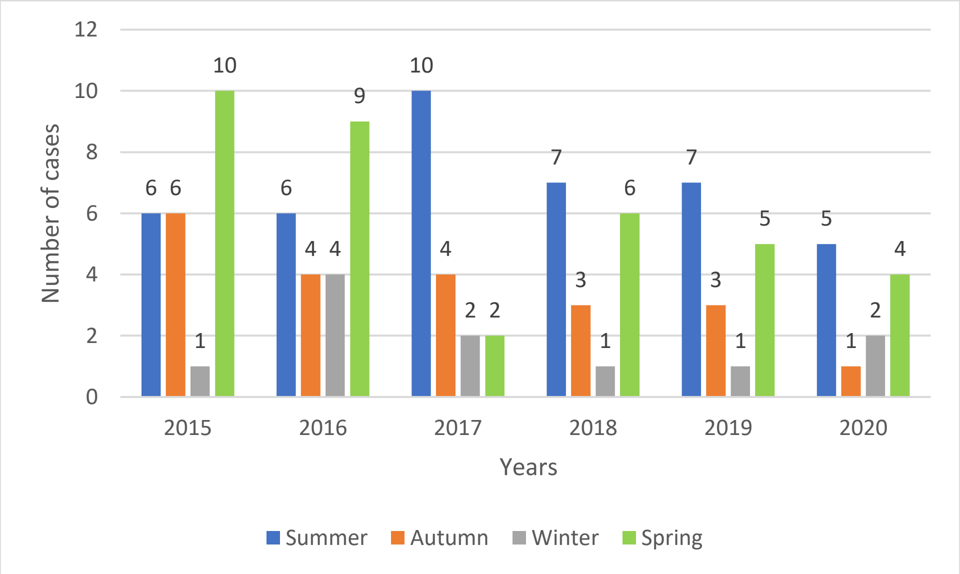

Figure 5: Seasonal distribution of cases with insect activity for the review period.

The most predominant form of insect activity noted on remains were maggots (n=104/109, 95.4%). Additionally eggs as well as other insects such as beetles, ants, cheese skippers, and other unspecified insects were noted in the cases.

With regards to insect activity and the different stages of decomposition (Figure 6), 68 of the early-stage decomposition cases (n=68/165, 41.2%) presented with insect activity, with the majority (58.8%) having no insect activity. For cases in the advanced stage of decomposition, the majority presented with insect activity (n=39/55, 70.9%). For cases in a skeletonised state, only two (n=2/6, 33.3%) presented with insects, which were beetles and fly larvae in the first case and only fly larvae in the second case.

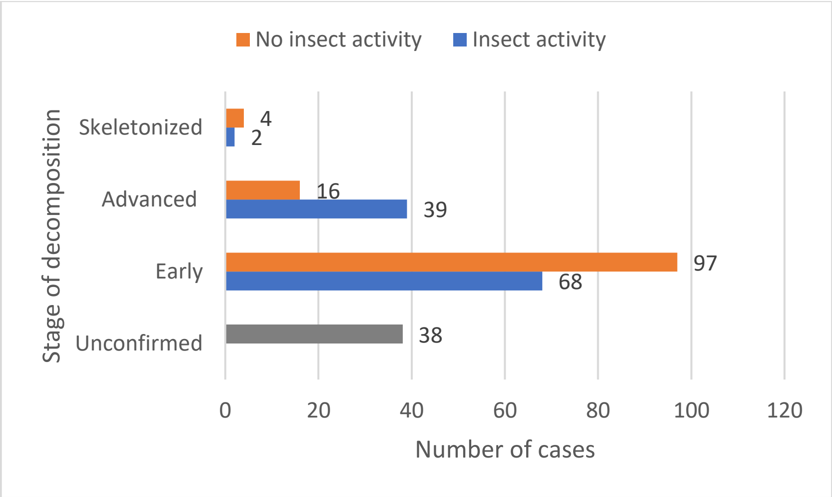

Figure 6: Stage of decomposition and insect activity.

2.3.1.4. Insect activity and type of scene

The cases that presented with insect activity were associated with various types of scenes (Figure 7). The proportion of indoor cases (n=135) that presented with insect activity was 42.2% (n=57/135) while the proportion of outdoor cases (n=129) that presented with insect activity was 40.3% (n=52/129). The difference was not statistically significant (p=0.75). Specific scene types associated with the most insect activity was formal housing (n=40/264, 15.2%) and farm/open land (n=32/264, 12.1%).

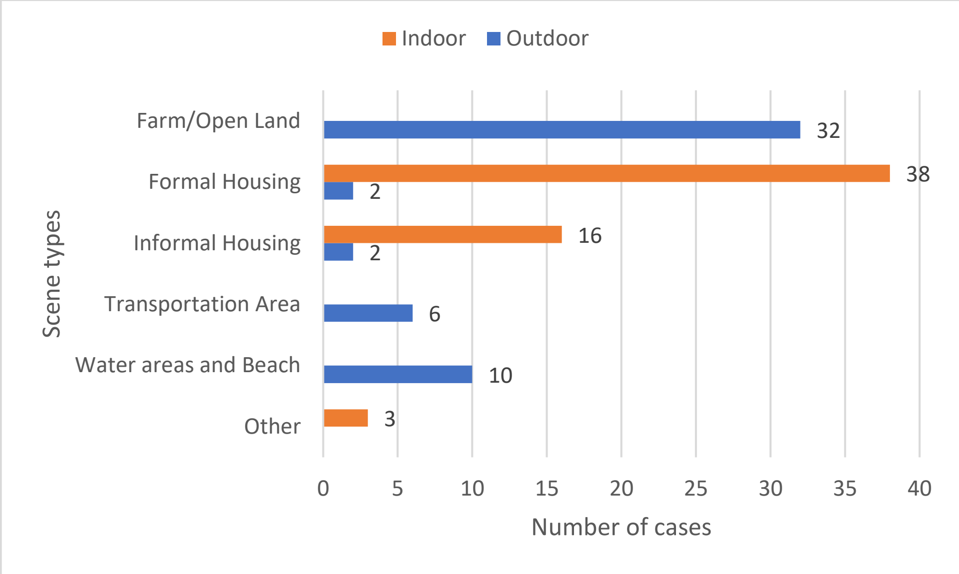

Figure 7: Type of scenes associated with insect activity.

2.3.1.5. Insect activity and condition of remains

The condition of remains with respect to covering (*e.g.*, buried, covered with materials or inside objects, submerged) and state of clothing, in relation to insect activity is shown in Figure 8. When ignoring clothing, the proportion of uncovered cases (n=159) that presented with insect activity was 54.1% (n=86/159) while the proportion of covered cases (n=67) that presented with insect activity was 34.3% (n=23/67). For cases where the remains were partially or fully clothed (n=162), insect activity was present in the majority (n=92/162, 56,8%). The inverse is true for remains that were unclothed (n=64), with most cases presenting with no insect activity (n=47/64, 73.4%). It should be noted that most unclothed decedents were foetuses or infants (n=41/64, 64.1%), of which 28 (n=28/41, 68.3%) had been concealed either by being wrapped in multiple layers, hidden in buckets or toilets, and hidden in drain canals along the street. These conditions may have limited insect activity due to difficulty accessing the remains.

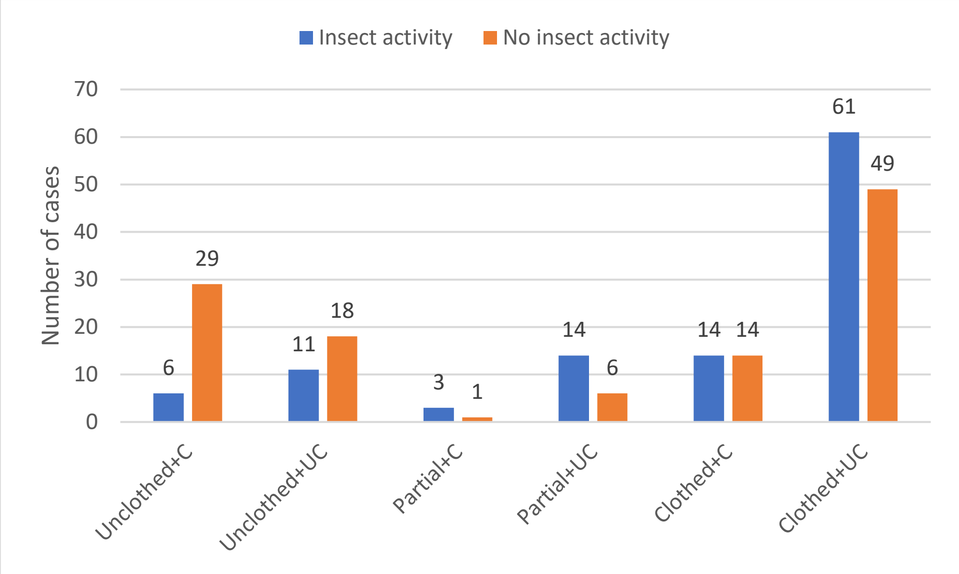

Figure 8: Insect activity associated with remains in various conditions of clothing and covering.

(Unclothed, partial, clothed refers to state of undress of remains) (C = covered either in form of burial, with materials, or submerged/ UC = uncovered)

2.3.1.6. Insect activity and trauma

The insect activity in relation to the presence of open wounds can be seen in Figure 10. When no open wounds were observed, most cases presented with no insect activity (n=77/137, 56.2%). These cases were mostly in early-stage decomposition (n=116/137, 84.7%) followed by advanced decomposition (n=19/137, 13.9%), and lastly skeletonised remains (n=2/137, 1.4%). When open wounds were observed, cases presented with and without insect activity in similar numbers, with insect activity being seen in 51.9% of cases (n=14/27). These cases were mostly in early-stage decomposition (n=21/27, 77.8%) followed by advanced decomposition (n=6/27, 22.2%)There were 50 cases where pathologists were unable to provide notes on open wounds, due to the poor conditions of the remains. Of these cases, most presented with insect activity (n=32/50, 64%), with one case file (2%) stating “excessive maggot feeding prevented any observation of open wound trauma”.

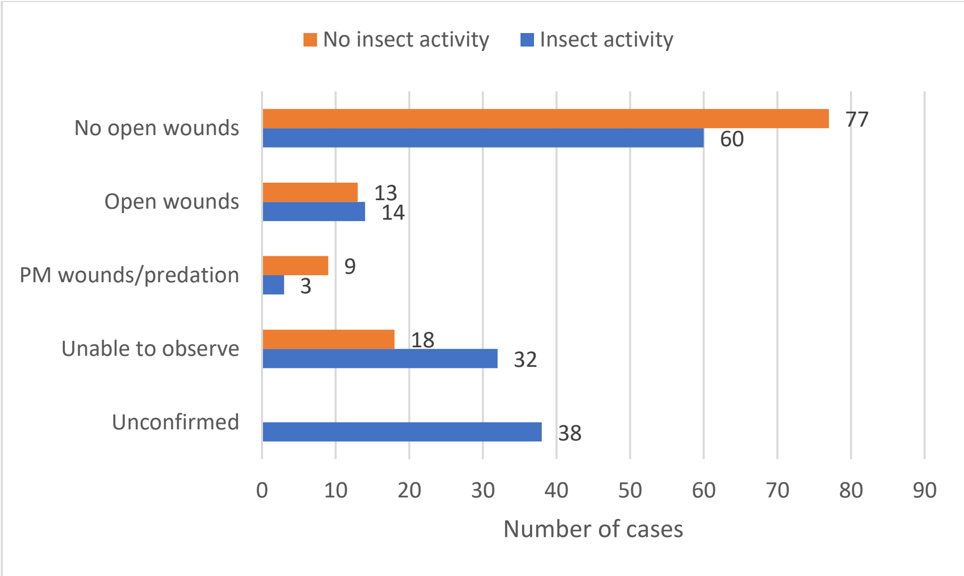

Figure 9: Insect activity associated with trauma.

2.3.1.7. Resources and methodology on the scene

With regards to documentation on scene, the forms used had varying degrees of completion between cases. The scene type or location is always noted on at least one of the documents in the case file, which may include an address or GPS location. The FPS002 document has space for scene sketches; however, this section was not utilised in all case files reviewed or was only used to refer to scene photography. Clothing and displacement thereof (if any) were noted in relevant sections. Contextual information was provided in the relevant sections, however, the amount provided varied between cases. Contextual information often included a recent history of the decedent (if known), conditions in which decedent had been found on the scene prior to law enforcement arrival (this included information regarding buried, submerged, or covered remains, as well as state of decomposition), and notes regarding insect activity or animal predation. Information that was not noted in scene documentation include weather conditions at the time of scene investigation, as well as various temperature measurements required for analysis of insect evidence.

2.3.2. Interview phase

For this phase of the study, forensic pathologists (FP's) and forensic pathology officers (FPO's) employed at Salt River mortuary were invited to participate in online interviews that made use of semi-structured questionnaires. Unfortunately, this phase of the study did not receive as much participant activity as expected, therefore thematic saturation may not have been achieved. Only two participants participated in the interview process, both of whom were Forensic Pathologists.

2.3.2.1. Procedures and documentation on scene

In close-ended questions regarding whether certain details are recorded during scene attendance, both respondents' answers typically aligned with one another (Table 3). Details about the scenes were always recorded (Yes=100%), along with various details regarding the decedent on the scene (Yes =100%). With regards to temperatures being recorded on scenes, the temperature of the decedent was recorded (Yes=100%) if the state of remains allowed it. Participants stated that no temperature recordings of insect masses were recorded on scenes (No=100%), while participants' answers differed with regards to surface temperature around remains being recorded (Yes=50%, No=50%). With regards to toxicological evidence, participants stated that the presence of this evidence is recorded (Yes=100%) while signs of drug use on the decedent are recorded if visible (Yes=100%). A remark by one participant was that only toxicological evidence related to the scene and decedent is documented.

Table 3: Responses to close-ended questions regarding data capturing on scenes.

	Type of data recorded	**Yes (%)**	**No (%)**
Scene:	Scene type	100	0
	Specific details about a scene	100	0
Decedent:	Position	100	0
	Clothing	100	0
	Coverings/buried or submerged	100	0
	Trauma/Predation	100	0
	Degree of decomposition	100	0
Temperature/Weather	Body temperature*	100	0
	Ambient temperature	100	0
	Insect masses	0	100
	Surface temperature (under remains)	0	100
	Surface temperature (around remains)	50	50
	Soil temperature	0	100
	Aquatic body/structure temperature	0	100
	Weather	100	0
Toxicology	Toxicology evidence present	100	0
	Signs of drug use**	100	0

* One respondent stated that if remains are too decomposed, temperature is not taken.
** Both respondents stated signs of drug use only recorded if visible (*i.e.*, needle marks).

All participants stated photography as a method of documenting the scene, with other methods including scene notes, sketches, context information (*e.g.*, received from officers on scene), and relevant measurements taken on the scene (includes temperature). One participant further stated that if photography cannot be done on scene (for various reasons), then photos are taken of the remains and any trauma, scavenging, or insect activity once at the mortuary. Furthermore, one participant stated that while individual insect masses may not always be separately documented in photos, it may be visible in photos that are taken of remains or the scene.

Participants could not provide information on how insect evidence is collected or documented on scene. Participants stated that collection of insect evidence could be delayed, or insect activity may be disturbed if the pathologist or pathology officer need to handle the remains in any way (e.g., remove wrappings around remains). If present, a forensic entomologist could be allowed to collect evidence before a pathologist or pathology officer tends to the remains.

2.3.2.2. Handing of remains and insect activity at mortuary and autopsy

Remains were always transported to and around the mortuary in body bags. With regards to remains that presented with insect activity, participants (n=2, 100%) stated that body bags can be sealed via stickers, but that the effectiveness of the stickers may decrease over time and result in insects escaping. One participant (n=1, 50%) had experienced insects escaping in cases they attended at the mortuary.

Participants stated that if insect activity is revealed during autopsies, it is documented in the form of notes or photography (100%). One participant stated that they have collected insect evidence from remains once it was documented, but provided no information as to how this was done (50%).

Participants stated the mortuary facility has insect traps (or insect killing devices) to prevent insects found within the building from colonising stored remains (100%), while one participant stated that keeping the body bag closed when in storage also ensured that insects from within the building did not colonise stored remains (50%).

2.3.3. Thematic analysis

Thematic analysis resulted in the identification of three themes relating to resources used on scenes or at the mortuary, knowledge and/or training of personnel attending the scenes, as well as scene-dependent variables that may affect handling of a scene. The themes and subthemes can be seen in Figure 10 .

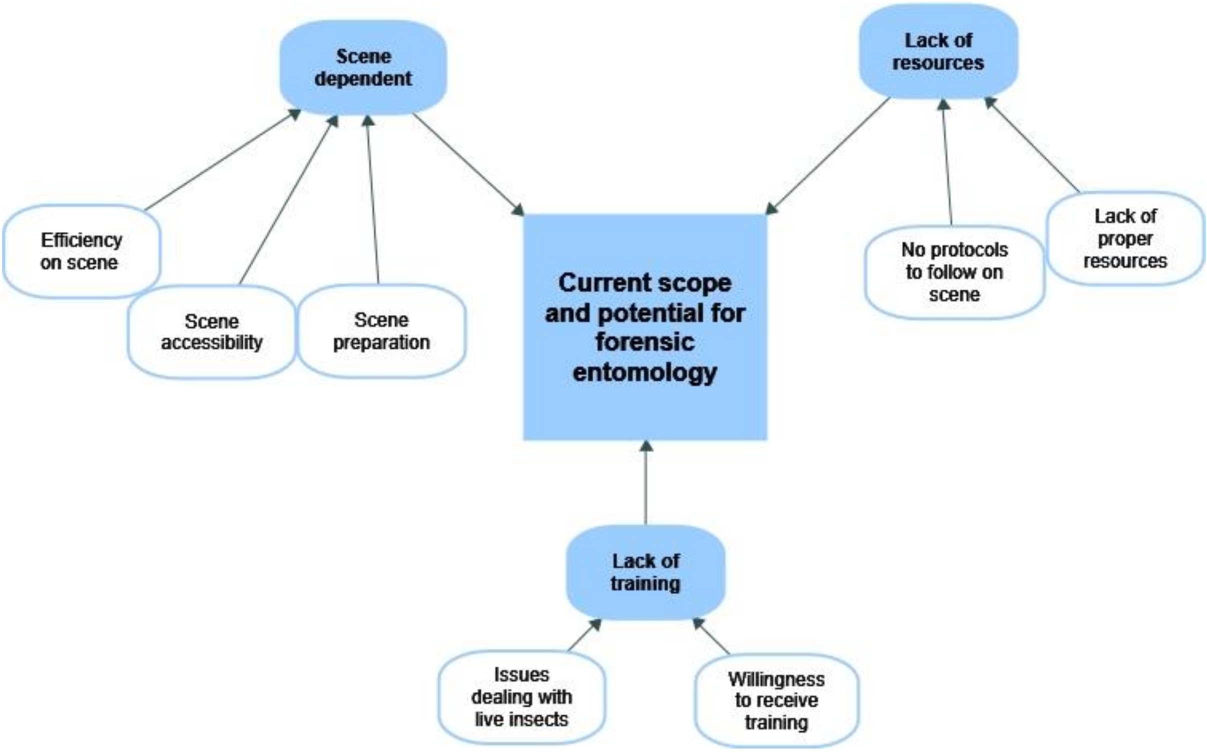

Figure 10: Thematic representation of the current scope and potential for forensic entomology at SRM.

2.3.3.1. Theme 1: Lack of resources

The lack of resources was highlighted by all participants as having a negative impact on the handling of a scene investigation. This includes the necessary tools to recover and handle evidence on scene, as well as protocols to follow when attending scenes that deal with decomposing remains and insect evidence. Participants noted that they make use of "what is available" to collect insect evidence (if done), and that it may not be "according to correct entomology practice". Furthermore, with no set protocol to follow with regards to entomology, participants will phone entomologists when on scene for assistance with handling any evidence.

2.3.3.2. Theme 2: Lack of training

In keeping with minimal resources, a lack of training with regards to handling and recovering entomological evidence was also highlighted by participants. Participants agreed that forensic entomology had its place in medico-legal investigations, but that a forensic entomologist was not always available to attend scenes. This issue was exacerbated by the fact that FPS personnel lacked knowledge on how to handle and recover insect evidence associated with human remains. Participants' answers indicated a willingness to receive training on how to handle and recover insects, despite some having "issues with live insects".

2.3.3.3. Theme 3: Scene dependent

A recurring answer during the interviews seemed to indicate that procedures or methods followed on scene tended to be "scene dependent". Time spent on scene was "scene dependent" or accessibility was "scene dependent". This is reflected in the review data as each scene is different to the previous and the next. This means challenges and issues on scenes may not be similar, as one participant recalls hiking to the scene while another remembers walking through shallow water to access remains. Participants stated that a call-out for scenes provides enough information for personnel to prepare, with one participant stating they "will ask questions for better understanding and to prepare".

2.4. Discussion

This study is one of the first in the Western Cape of South Africa, providing insight into the scope of death scene investigations associated with decomposing human remains and insects. The study provided a review of cases associated with insect evidence for the period 2015 to 2020, as well as insight from FPS personnel into the procedures used when dealing with cases involving entomological evidence, and any issues or challenges that they may face.

Internationally, there are standardised protocols set up by forensic entomologists of the European Association of Forensic Entomology (EAFE) (Amendt *et al.*, 2006) and the American Board of Forensic Entomology (ABFE) (Byrd *et al.*, 2010) that are used during medico-legal investigations in their forensic environment. Currently, there is no standard protocol being used by FPS in South Africa, which could assist in any case associated with insect evidence in the absence of a forensic entomologist.

The findings of this study highlight the need for training of personnel and the development of a standard procedure that can be followed when attending scenes associated with insects. Furthermore, information was gathered regarding what cases may likely present with insects, as well as the issues and challenges associated with these cases. This information can assist in preparing personnel for what evidence may be present on scenes and could be used to assist in the development of a standard procedure.

2.4.1. Insects and death scenes

A review of decomposition cases for the period 2015 to 2020 saw 264 cases that fit the criteria of the study. Decomposition cases were chosen for the study because these cases are investigated when an unknown amount of time has lapsed since death, meaning more conventional means of estimation PMI (body changes or temperature) may no longer be useful. This is where the presence of insects will assist in the investigation, as decomposing remains tend to attract insects far more than fresh remains. While the confirmed cases that presented with insect activity was less than half of the decomposition cases (n=109/264, 41.3%), it is still a large number that suggests there is a place for entomological analyses in death investigations. There is no confirmation as to how many of the reviewed cases were sent for entomological analyses, as entomological reports were not present in the case files except for one. Unpublished data from Adetimehin (2023) suggests few cases over the review period have been sent for entomological analysis.

When attending scenes, SRM personnel make use of forms (*e.g*., Lab 27, FPS002) to document details regarding the scene, the decedent(s), and any information that might be pertinent to the death investigation. These forms are typically completed to the best of the FPO or attending pathologist's ability, with some information being supplied by other personnel on the scene (*e.g*., police officer). None of these forms have any sections pertaining to specific information regarding a decomposition cases or insect activity that might be present on scene. This information, if added, is typically found in a "additional notes" section.

2.4.1.1. Seasonality and weather

The retrospective data gathered in this study helped provide a bigger picture with regards to decomposition cases and associated insects. At the outset, it is interesting to note that decomposition cases for the year 2020 was the highest for the review period (n=53/264, 20.1%) with little fluctuation between the seasons (Figure 1). The year 2020 was also the year with the lowest number of cases assigned to SRM in the review period (n=3495 with an average of 3830 cases over the review period). In 2020 the globe was marred by the COVID-19 pandemic and subsequent lockdowns and restrictions. South Africa went into the first hard lock in March 2020. This was only lifted in 2022, and during this time various levels of lockdown and restrictions on movement were implemented. The hard lockdown impacted crime and murder numbers in the initial months of 2020 (Kelly *et al.*, 2021), which may have decreased the number of cases seen at SRM, while the higher number of decomposition cases could be explained by lockdown impacting travel and potentially limiting the discovery of any decedents living alone or in hidden or secluded areas. Despite the higher number of decomposition cases for 2020, the insect activity seen in cases followed the same trend as previous years (Figure 5).

The cases associated with insects were not subject to one or a few seasons in a year, but rather fluctuated throughout a given year. Warmer seasons, such as summer and spring, tended to have higher numbers of cases presenting with insect activity (Figure 5), with the winter season having the lowest number of cases. This correlates with what may already be known about forensically important insects in South Africa and other countries in the world – different insects present with different seasonal activity (Patitucci *et al.*, 2011; Dufek *et al.*, 2019). For example, a study by Tembe and Mukaratirwa (2021) saw flies such as *Musca domestica* (Diptera: Muscidae), *Chrysomya albiceps* and *Chrysomya marginalis* (Diptera: Calliphoridae) more active in the warm season compared to the colder season, while a species such as *Antherigona soccata* (Diptera: Muscidae) only appeared in the colder season.

One concern about weather and its impact on insect activity is extreme temperatures, especially in recent years due to climate change associated with global warming. A study by Williams and Villet (2019) saw one species of fly that was typically active in winter suddenly become absent in bait traps due to excessively low temperatures. The data indicate that insects may not be as active as expected when temperatures are unusually cold or hot, which may become more common due to climate changes.

Weather conditions may further play a role in the rate of decomposition, and thus the rate of insect colonisation. While warm weather typically allows for faster decomposition and thus attraction of insects to the remains, studies have shown that certain weather conditions (e.g., unexpected rainfall during warmer seasons) may slow the decomposition down and may even act to wash away insect eggs or larvae present on the remains (Lyu *et al.*, 2016; Singh and Bala, 2019).

According to the Western Cape Government, the Western Cape will be experiencing many affects in years to come due to climate change, which includes higher maximum and minimum temperatures, reduced average rainfall, and an increase in the frequency and intensity of extreme weather events (Western Cape Government, 2022). The expected climate changes, along with data from studies like those of Williams and Villet (2019), Lyu *et al.* (2016), and Singh and Bala (2019), suggests the need for continuous research into the minimum and maximum temperatures at which forensically important species may be active. Furthermore, the studies indicate the need for caution when dealing with cases that may have had unusual weather conditions affect insect activity. Overall, it is best to ensure weather conditions and temperatures are properly recorded when attending death scenes presenting with insect activity.

2.4.1.2. Scene type

As with seasonality, the type of scene in the current study had no strong influence on the presence of insects either. The data indicated that indoor and outdoor scenes presented with similar rates of insect activity (42.2% and 40.3% respectively) while two specific scenes (farm/open land and formal housing) were the most common scenes associated with insect activity. A retrospective study meant that identification of insects at these scenes could not be done, however, other identification studies have shown why certain scenes may seem more favourable for insect colonisation compared to others. Dufek *et al.* (2019) and Williams and Villet (2019) suggest that insect species will be abundant in areas where they may find favourable food sources and microhabitats, with these areas typically being human-rich or land with crops and/or animals. Furthermore, Kavazos and Wallman (2012) suggest that weather may play an additional role in the areas that insects will be more abundant or active in, with insects being active in human-rich areas while being inactive in more natural sites during the same seasonal period. Certain species may prefer spending cooler seasons in human-rich areas where they can easily access food sources and cover from the weather. During warmer seasons, insects may typically be less abundant or perhaps less active in areas that consist of mostly open land since there is less cover to take shelter from extreme temperatures (Reid and Hochuli, 2007). Areas that provide insects with abundant food sources and cover from weather conditions will have a high abundance of insects, meaning a higher chance of decomposing remains attracting and being colonised by insects.

2.4.1.3. Condition of remains

Other factors besides weather and scene location can affect how and when insects colonise the remains. Firstly, decomposition happens in stages and each stage attracts its respective share of insects. Remains are highly attractive to certain insects during the first few hours or days of decomposition (Williams and Villet, 2019), otherwise known as the fresh and early stages of the decomposition process (Galloway, 1997). Of the cases reviewed in this study, the proportion of early-stage decomposition cases that presented with insect activity was 41.2% while the proportion of advanced stage decomposition cases presenting with insect activity was 71.4%. (Figure 6). This may suggest that the longer the remains are available, the more opportunity there is for insect colonisation. As this was a retrospective review, identification of insects during these stages could not be done, but the data fall in line with other studies (Tembe and Mukaratirwa, 2021) that show increased insect activity during the early to advanced stages of decomposition. This increased activity is due to remains becoming more attractive as a food source and oviposition site for insects, as soft tissue starts breaking down and odours begin emanating from the remains. Furthermore, some research has stated that feeding larvae could influence further colonisation of the remains by opening the body as feeding continues, releasing odours that attract other species not yet present (Martin and Verheggen, 2018). This phenomenon of multiple species colonising the same remains or oviposition site is to increase survivability of larvae, as bigger maggot masses produce the optimum temperatures for larval development, and secretion of digestive enzymes by older larval stages will aid younger larvae as they feed on remains (Rivers *et al.*, 2011; Martin and Verheggen, 2018).

Remains that are covered – in other words not exposed to the environment - may also affect how insects colonise them. Depending on how remains are covered or clothed, the decomposition odours could be prevented from escaping and thus attracting insects, while the materials used may limit the available oviposition sites for females.

The clothing or covering of remains may therefore act to slow down insect colonisation. However, as represented by the data in Figure 8, covered remains may still attract insects, but it occurs less frequently in comparison to the cases in which remains were uncovered. The data suggest that colonisation may occur faster when remains are open to the environment, or are loosely covered (i.e., not wrapped tightly with gaps for insects to move through). A study by Kelly *et al.* (2009) documented that insects were attracted to covered remains and uncovered remains in the same time frame, but noted that oviposition may be delayed in the former if the remains were thoroughly wrapped and insects had to crawl around to find suitable sites. Another study by Card *et al.* (2015) observed that covered or clothed remains may actually provide female insects with more oviposition sites, as eggs were found in the folds of clothing and waistbands and collars. While insect attraction and succession may be similar between covered and exposed remains, Kelly *et al.* (2009) observed that covered remains were in the advanced stage of decomposition longer than exposed remains, suggesting that wrapping or covering remains will delay the decomposition to a certain degree. This leads to the suggestion that the analysis of insect evidence may be the more reliable route when determining a post-mortem interval.

The degree of clothing in which remains were found seemed to play a small role in insect activity at the scene, since a higher rate of insect activity was observed in cases where remains were mostly clothed compared to partially or unclothed remains (Figure 8). As stated, most unclothed decedents were foetuses or infants (n=47/64, 73.4%) with 28 (n=28/41, 68.3%) of these cases being concealed in some way (hidden in buckets, toilets etc.). Most of these cases (n=22/28, 78.6%) were in the early stage of decomposition, suggesting that concealment may have slowed down the rate of decomposition, which may in turn have decreased the rate of insect colonisation. Research shows that insects typically deposit eggs in the eyes, nostrils, and mouth to provide larvae with easier access to mucus membranes and softer tissue for feeding (Brodie *et al.*, 2015), indicating that remains do not need to be unclothed for it to be colonised. Furthermore, oviposition may occur around or near remains that are buried or covered. A study by Bhadra *et al.* (2014) observed flies laying their eggs on zippers that were in contact with either tissue or just blood, as the insects were attracted to the odours or bodily fluid. This data is important as it suggests that remains in body bags could attract and therefore be colonised by new insects that are not already present, however, the chances of this are lowered if the transport of bagged remains from scene to mortuary is conducted swiftly (Bhadra *et al.*, 2014).

When it comes to the impact of open wounds, this study's six-year review period showed there was no significant difference in insect activity when the cases presented with or without open wounds (Figure 9). The cases that presented with wounds were mostly in the early stage of decomposition and a few cases in the advanced stage, with insect activity being seen more frequently in the early decomposition cases. The cases that presented with no wounds were mostly in the early stage of decomposition, followed by advanced decomposition cases and two skeletonisation stages. Thus, the unexpected finding of no association with the presence of wounds may have been compounded by the stage of decomposition. Many experts and investigators think open wounds would provide more attractive sites for oviposition, as hatching larvae will have easier access to a food source (Charabidze *et al.*, 2015). However, the presence of larvae in a wound does not imply that the eggs were laid in that exact wound. This is the observation made by Charabidze *et al.* (2015), as no eggs were being laid by necrophagous flies in the experimental wounds on rat cadavers, but rather in the usual oviposition sites. Another study by Kelly *et al.* (2011) observed that regardless of the presence of wounds, all oviposition sites remained the same - ears, nostrils, and mouth. Much research has been done to understand why and how egg-laying insects choose oviposit sites. Insects may choose sites depending on how much food is available and how far larvae may need to travel to access the food source (Schwartz *et al.*, 2012). Insects may further choose not to lay eggs in a site that is too small, as the survival rate of eggs and larvae may be compromised by depleting oxygen levels (Archer and Elgar, 2003), or they prefer sites like the ears, nostrils, and mouth over wounds to ensure eggs are not saturated or washed away by bodily fluids (Kelly *et al.*, 2011). Since the oviposition behaviour of insects differs between species, and other factors also play a role in how and when insects are attracted to human remains, it cannot be strongly concluded that the presence of wounds will increase the chance of insect colonisation.

2.4.2. Prospective data

While the retrospective study suggested that forensic entomology can and should be implemented more actively during medico-legal investigations, the data gathered from interviews provided insight and recommendations into how it can be implemented. The data collected were limited due to a small response from intended participants. The data was expected to be skewed, especially since the participants were of the same occupation (forensic pathologist) and stated that they did not attend all scenes assigned to Salt River. However, the data still holds value and should be analysed, as they may highlight areas that may be open to research or issues to consider when implementing forensic entomology at a mortuary.

One major issue mentioned by all participants was that there is a lack of training for both FPO’s and pathologists with regards to forensic entomology and the handling of the respective evidence. This indicates that personnel attending scenes associated with insects may not be able to handle insect evidence correctly, which could create downstream issues should a forensic entomologist be called for their opinion later. Unfortunately, forensic entomologists are not readily available to attend scenes and contacting one while on the scene might be impossible. It was therefore suggested by participants (n=2, 100%) that training courses be provided to improve the knowledge of those attending death scenes with insect activity. It was further suggested by one participant that if no proper protocol is implemented, then a simple-to-follow diagram on how to sample insects be made for personnel attending the death scenes. Recovery of insect evidence done on the scene will ensure that no issues will arise, such as missing any developmental stages of the insects or certain species (Lutz *et al.*, 2021), while having knowledge and a proper process to follow will prevent any improper handling or contamination (Archer *et al.*, 2006; Archer *et al.*, 2018).

Along with lacking training and knowledge, participants also indicated that resources typically required for collecting evidence on scenes (e.g., specimen vials, swabs) may not be sufficient (n=2, 100%), which is currently a common issue in the country. Participants went so far as to state that they would make use of “what is available” to recover insect evidence. This means the recovery may not be according to proper entomology practice, which could create issues in the analysis of the evidence. The issue of insufficient resources and its consequences, or even the misuse of available resources, will need to be considered if implementing forensic entomology as a common practice in death scene investigations, as it could mean issues further down the road when considering the reliability of the collected insect evidence.

Participants also indicated that scenes differ from one another and that could affect how each one is handled. Alone, varying conditions on scenes might not seem like a problem, but together with the issues of insufficient training and resources, could become a bigger problem. To put it simply, the lack of simple training and insufficient resources could mean the method of collecting evidence on one scene would differ from another. Analysis of the collected evidence from the different cases may prove problematic and the different recovery methods might be frowned upon if brought to court.

Overall, two of the main themes identified during the prospective study – lack of training and knowledge, and poor resource availability and management – come together to form issues seen not only in entomological cases, but in many legal cases in South Africa. A study by Visser and Oosthuizen (2009) highlighted an issue regarding DNA evidence at the Forensic Science Laboratory in South Africa – many samples submitted for analysis are not analysed as a result of the poor collection methods used. Furthermore, the lack of proper training on how to handle and recover evidence on scenes, and thus the knowledge on how to identify said evidence, leads to officials missing reliable evidence that would otherwise have been valuable during a criminal trial (Visser and Oosthuizen, 2009). The lack of training, or perhaps poor training, of officials in the forensic field in South Africa seems to be an ongoing issue, and this study has provided evidence to that fact with regards to forensic entomology. Highlighting these issues now is vital, so that when forensic entomology is implemented on a greater scale in death investigations, it will be done so in the best possibly capacity. With regards to resources, evidence collection kits have been developed over the years to aid in evidence recovery, such as the Sexual Assault Evidence Collection Kits (SAECKs). Kits like these could become common tools utilised on scenes and could even be developed with focus on entomological evidence, however, it has been stated that kits like the SAECKs are not always used to their full capacity (i.e., missing specimens) (Visser and Oosthuizen, 2009), and in some cases the kits are not sent to the Forensic Science Laboratory for analysis (Machisa *et al.*, 2017). Furthermore, there are cases in which forensic nurses or medical doctors are required to collect evidence using kits supplied by the South African police, but many of the cases lack evidence collection due to police failing to supply the kits or case numbers (Abdool and Brysiewicz, 2009). While specifications for an entomology evidence collection kit (ENT) have been outlined by SAPS, it is not confirmed whether such a kit has been developed for use on scenes. Regardless, the issues highlighted with other evidence kits will need to be considered during the development or subsequent implementation of any entomological evidence kits.

2.4.3. Recommendations and study limitations

Several limitations should be considered in interpreting the results presented in this dissertation. The retrospective phase may be limited by missing or incomplete documents leading to incomplete data collection. Furthermore, retrospective studies provide no insight into what exactly is done on these scenes and where issues may arise. However, this does not downplay the data generated, as it does provide insight into the scope of cases associated with insect activity and the potential for forensic entomology to be implemented more actively. The development of the new Observatory Forensic Pathology Institute (OFPI) provides the capacity for a dedicated forensic entomologist or entomology unit to be employed, thus providing more assistance in estimating post-mortem intervals for medico-legal cases and allowing FPS to have more control over forensic entomology used in cases while ensuring proper resources are available. Due to the many uses of forensic entomology, it is unclear whether forensic entomology should fall under the purview of a single entity such as SAPS or FPS. More research should be conducted to determine whether forensic entomology utilised by SAPS and FPS can be combined into one unit, with multiple subunits working on either reconstruction of death or the estimation of time of death respectively. Further studies into the scope of cases associated with insects would provide even more insight into how to implement forensic entomology at this level. These studies may be retrospective, but it is recommended to either widen the review period (which went beyond the resources of this study) or doing a prospective study to allow for observations on the scenes and data to be collected about processes on the scenes (not possible for this study due to the COVID-19 pandemic and restrictions). Furthermore, as this study did not analyse scene photos, prospective studies might assist in evaluating whether photo documentation on scene is sufficient or requires improvement.

With regards to the interview phase, the data were limited due to a very small number of participants agreeing to the interviews. This may be for number of reasons, but it could mean the data collected are skewed to a certain viewpoint, especially in this study due to all participants being forensic pathologists. However, the data can be seen as a starting point to gain further insight into what personnel may experience when attending scenes or their experience with insects on these scenes and at the mortuary. It further provides insight into how forensic entomology and the practice thereof can be implemented and even improved at OFPI in the future, as participants themselves provided recommendations during the interviews. Further research would provide more participants and thus viewpoints with regards to forensic entomology and its implementation, and it may be best to make it an online survey to allow for more participants (as busy schedules may have influenced participation) as well as expanding the participant pool to include other mortuaries in the province or country.

2.5. Conclusion

This study made use of a retrospective review and interviews with personnel to gather data on the current scope of forensic entomology and decomposition cases. Issues and limitations that personnel face when dealing with cases presenting with insects were also highlighted. While this study revealed that insects could be present in a variety of cases with different circumstances, it also helped identify what improvements may need to be made to handle these scenes efficiently. Currently, the poor recovery of insect evidence is due to the lack of training and possibly poor availability of resources. This highlights the need to train personnel that attend death scenes associated with insects, as well as research into what exactly is available on scenes for evidence recovery and how to improve these resources and the availability thereof. This could lead to the standardisation of forensic entomology practices in South Africa.

This study provides a clear indication that there is a place for forensic entomology in medico-legal cases and highlights the gaps that need to be filled to facilitate the use of forensic entomology. The first recommendation would be the development and implementation of a standard protocol, to be used on scenes presenting with insect activity. This will ensure personnel know what to do on scenes when an entomologist may not be present. Furthermore, continuous training of personnel should be implemented, to ensure they remain informed about current research and methodologies in the entomology field. Lastly, the development of an entomology evidence collection kit may further assist personnel on scenes, as it would make the recovery and handling of entomological evidence more efficient and may be of great value when considering volatile scene environments. Lastly, more research into entomological cases in South Africa will need to be done as this study only focused on the West Metropole of the City of Cape Town and may not reflect entomological cases and circumstances throughout the country.

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APPENDICES

Appendix A: Interview Questionnaire

Data collection regarding entomological evidence

Estimated time spent on scene: ___________________________

How many FPO on scene: _______

If pathologist on scene, how many:________

Scene of death	
Details of scene taken:	Yes/No
If outdoor scene, specific details of environment taken:	Yes/No
If indoors, specific details of setting taken:	Yes/No
If indoors, details of floor taken:	Yes/No
Details of heating, cooling, windows taken:	Yes/No
If aquatic scene, specific details of environment taken:	Yes/No
If other scene setting (e.g., car) is information of setting taken:	Yes/No
Details of scene given to FE if one is not present:	Yes/No
Remarks:____	

Specifications	
Details of position of remains taken:	Yes/No
Details of clothing on remains taken:	Yes/No
Information on any other covering, if present, taken:	Yes/No
Degree of decomposition noted:	Yes/No
Details of potential wounds or scavenging taken:	Yes/No
Remarks:____	

Temperature & Weather Data	
Temperature of body recorded:	Yes/No
How?____	
Ambient temperature (air around body) recorded:	Yes/No
How?____	

Surface temperature under the remains recorded: Yes/No

How?____

Surface temperature away from remains recorded: Yes/No

How?____

Temperature of soil (~20cm below remains) recorded: Yes/No

How?____

Temperatures of maggot masses recorded: Yes/No

How?____

Temperature of structure/aquatic body recorded: Yes/No

How?____

Details of weather taken: Yes/No

Toxicology

Details on any toxicology evidence present on scene taken: Yes/No

Details on any signs of drug use on body taken: Yes/No

How? ____

____

Documentation

Photography ☐ **Videography** ☐ **Sketches** ☐ **Notes** ☐ **Context** ☐ **Measurements** ☐

Are photos taken of the scene: Yes/No

Are photos taken of the remains: Yes/No

Are photos taken of signs of trauma or scavenging on remains: Yes/No

Are photos taken of insect activity (maggots on/around/near body): Yes/No

Remarks : ____

____

____

____

If insect evidence is also collected on scene, in what order is it done (list below in order e.g., 1st – scene details, 2nd - flying insects, 3rd – temperatures, 4th - maggots, 5th - remains, 6th - any insects on ground/below body etc.):

____

____

____

Is sampling of all insect activity performed or only a few select sites?

With decomposed remains, is there any evidence that must be collected before insect evidence can be collected/may have affected collection of insect evidence?

At the Mortuary

How are remains removed from vehicle:

Placing & storage of remains (are body bags left open, semi-open etc.):

Is storage temp known: Yes/No

At the Autopsy

If the remains are decomposed but insect activity is only revealed at autopsy (i.e., opening of body reveals insects inside remains), what is done?

Tools/Resources Checklist		
Tool/Resource Name	**Yes**	**Comment**
Sterile gloves		
Small paint brush (collect eggs)		
Small spoon (collect larvae)		
Forceps (collect larvae, pupae, adults)		
Near-boiling water source (kill larvae)		
Screw-topped vials + ≥80% ethanol		
Blank labels for inside the vials		
Insulated box (transport live samples)		
Ventilated rearing vials (transport live specimens)		
A lead pencil for labelling		
Blank labels for ventilated vials.		
Shovel/trowel (to take soil and leaflitter samples and search for buried insects)		
Resealable plastic or robust paper bags (transport soil and leaf litter samples)		
Standard entomological evidence forms/sketches (record the origin of each sample and the associated conditions)		
Standard chain-of-custody form for insect evidence		
Scene indicator flags (indicate location of insect evidence in photographs)		
HD video camera (record general site conditions and specific details)		
Digital thermometer (measure temperatures within maggot masses)		
GPS receiver (establish accurate location data that may help to establish the aspect of exposure of the corpse, times of local sunrise and sunset, and the distance(s) to the weather station(s) used in the investigation)		
Electronic data loggers, pre-set to record temperatures to match local weather station recording intervals (to determine temperatures on site after body recovery, which can be used to estimate temperatures before body discovery.		

Semi-structured questionnaire

1) Does the call-out for scenes provide enough information for preparation (e.g., knowing what to expect, what equipment to take with)?

____

____

2) Do you ever experience issues with getting to a scene (e.g., difficulty navigating areas such as informal settlements/open environments etc.)?

____

____

____

3) How close to a scene are you typically able to park the vehicle?

____

____

4) Is a forensic entomologist called out to all scenes involving insects?

____

____

5) If no entomologist is present, do you have the necessary tools to collect insects and any additional information for the entomologist?

____

____

6) Does the mortuary have protocols in place that assist with cases that involve insects associated with decomposed remains?

____

____

7) **With no FE present:** On a scale of 1 to 5, how helpful are the present protocols for dealing with the insect activity?

____

_____

8) On a scale of 1 to 5, how necessary do you think the entomologist is on the scene (1 = not necessary, 5 = very necessary)?

____

9) Do you feel comfortable/prepared to handle collection of insect evidence?

____

10) Do you think collection of insect evidence and associated information is necessary?

____

11) Do you find that there are challenges or issues with handling decomposed remains that have insect activity on the scene or at the mortuary? Please explain

____

12) In your opinion, what are some of the limitations that prohibit you from collecting insect evidence and additional information? (e.g., no tools, no knowledge on how to do it, disturbed scene etc.)

____

13) Would further training with regards to handling of insects be useful? How often should training occur if yes?

____

14) Does a lack of resources have a negative impact on collecting information that will be necessary for the pathologist/entomologist?

____

15) **Transportation of decomposed remains**: how are body bags sealed, and are they sealed well enough to prevent escape of insects?

____

____

16) How does the mortuary prevent the colonisation of stored human remains with insects found within the building (i.e., prevent flies found in the building from infesting and laying eggs in a recently arrived body)?

____

____

____

17) Can you provide any other challenges or issues, or even a different method of handling the remains and insects on scene or in the mortuary, which has not been covered by previous questions?

____

____

____

____

Appendix B: Ethics Approval and Extension Letters (HREC Ref: 249/2021)

UNIVERSITY OF CAPE TOWN

Faculty of Health Sciences

Human Research Ethics Committee

Room 45, E-52- Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: hrec-enquiries@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

29 November 2021

HREC REF: 249/2021

Mr C Mole
Division of Forensic Medicine & Toxicology
Falmouth Building
Email: calvin.mole@uct.ac.za
Student: lbstyr001@myuct.ac.za

Dear Mr Mole

PROJECT TITLE: PROTOCOL DEVELOPMENT FOR RECOVERY AND HANDLINGOF FORENSIC ENTOMOLOGY AND TAPHONOMIC EVIDENCE DURING DEATH SCENE INVESTIGATIONS. MPHIL CANDIDATE: MS T LAUBSCHER

Thank you for your response letter, addressing the issues raised by the Faculty of Health Sciences Human Research Ethics Committee (HREC).

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

This approval is subject to strict adherence to the HREC recommendations regarding research involving human participants during COVID -19, dated 17 March 2020: 06 July 2020 & 01 July 2021.

Approval is granted for one year until the 30 November 2022.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

The HREC acknowledge that the student: - Ms Tyrian Laubscher will also be involved in this study.

Please quote the HREC REF 249/2021 in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

HREC/REF 249/2021sa

Yours sincerely

PROFESSOR M BLOCKMAN

CHAIRPERSON, FACULTY OF HEALTH SCIENCES HUMAN RESEARCH ETHICS COMMITTEE

Federal Wide Assurance Number: FWA00001637.

Institutional Review Board (IRB) number: IRB00001938

NHREC-registration number: REC-210208-007

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2020), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI), and Declaration of Helsinki (2013) guidelines. The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

HREC/REF 249/2021sa

UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938)			
This serves as notification of annual approval, including any documentation described below.			
☑ Approved	Annual progress report	Approved until/next renewal date	30/11/2023
☐ Not approved	See attached comments		
Signature Chairperson of the HREC/ Designee		Date Signed	22/11/2022

Note: Please email this form and supporting documents (if applicable) in a combined pdf-file to hrec-enquiries@uct.ac.za.
Please clarify your plan for research-related activities during COVID-19 lockdown.
Please use the latest form found on our website:
http://www.health.uct.ac.za/fhs/research/humanethics/forms

Comments to PI from the HREC

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	11 November 2022		
HREC REF Number	249/2021	Current Ethics Approval was granted until	30 Nov 2022
Protocol title	Protocol development for recovery and handling of forensic entomology and taphonomic evidence during death scene investigations		
Protocol number (if applicable)			
Are there any sub-studies linked to this study?	☐ Yes	✓ No	
If yes, could you please provide the HREC Reference number for all sub-studies? Note: A separate FHS016 must be submitted for each sub-study.			
Principal Investigator	Calvin Mole		

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

Appendix C: Ethics approval - Amendment to staff (HREC REF: 528/2021)

UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

Form FHS007: Amendment – study staff

HREC office use only (FWA00001637; IRB00001938)			
☑ Approved			
This serves as notification that all changes to the study staff and documentation described below are approved.			
Chairperson of the HREC signature/ Designee	[signature]	Date	22/11/2022

Note: Please note that incomplete amendment submissions will not be reviewed.
Please email this form and supporting documents (if applicable) in a combined pdf-file to hrec-enquiries@uct.ac.za.

Please clarify your plan for research-related activities during COVID-19 lockdown.

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	18 November 2022		
HREC REF Number	528/2021		
Protocol title	Insect succession and changes in the soil pH and electrical conductivity associated with decomposing pig carcasses on the Table Mountain National Park of the Western Cape Province of South Africa		
Protocol number (if applicable)			
Principal Investigator	Calvin Mole		
Department / Office Internal Mail Address	Department of Pathology, Division of Forensic Medicine and Toxicology Falmouth building Entrance 2 Level 5, Rm 5.10		
1.1 Does this protocol receive US Federal funding?		☐ Yes	✓ No

2.1 Staff changes (tick ✓)

Are new personnel being added to this research?	✓ Yes	☐ No
Are current personnel being removed from this research?	☐ Yes	✓ No

16 February 2022 Page 1 of 2 FHS007

HUMAN RESEARCH ETHICS COMMITTEE
21 NOV 2022
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

Is the principal investigator for this research being changed? If yes, please attach revised conflict of interest and PI declaration statements. (Refer: sections 7 and 8.3 in the New Protocol Application Form - FHS013)	☐ Yes	✓ No
Do the consent and assent forms need modification to reflect these staff changes? If yes, please attach copies of the revised forms, with all changes highlighted or tracked and listed in the documents for approval.	☐ Yes	✓ No

2.2 Amended study staff details

Title, first name, surname	Department/Division	E-mail	Role of new staff member
Ms Tyrian Laubser	Pathology/Forensic Medicine	LBSTYR001@myuct.ac.za	Data collection and analysis

3. List of documentation for approval

Please list below all staff documentation such as CVs, declarations, GCP certificates and revised consent forms which need approval. This information must correspond to all 'yes' answers in 2.1 above. This form will be signed and returned to the PI as notification of approval. Please add extra pages if necessary.
N/A

4. Signature

My signature certifies that I will maintain the anonymity and/ or confidentiality of information collected in this research. If at any time I want to share or re-use the information for purposes other than those disclosed in the original approval, I will seek further approval from the HREC.			
Signature of PI		Date	18 November 2022

Appendix D: Consent form

CONSENT FORM PID__________

I, ______________________________ (full name, block letters) am a forensic pathology officer (FPO)/Forensic Entomologist/Forensic Pathologist at the Salt River Pathology Laboratory/CapeFORTE (circle those that apply):

I accept to be a participant of this specific research study	
Yes	No
I have been informed that I will be interviewed on what is done on a scene involving decomposed remains and associated entomological evidence, as well as any challenges, risks or issues faced during the scene visit and investigation, for research purposes. I have also been informed that the interview may include questions regarding the handling of the remains in the morgue.	
Yes	No
I have been given the opportunity to ask questions about the study and any misunderstanding I may have had, has been addressed.	
Yes	No
I understand that all data collected during this interview with participants will be used to improve the recovery, handling, documentation, and transport of decomposed remains and associated entomological evidence.	
Yes	No
I accept that all data collected during these interviews may be used for further research into the designing of protocols.	
Yes	No
I understand that the researcher wishes to publish the data once confidentiality has been ensured.	
Yes	No
I grant permission for the interview to be recorded during its duration (recordings to be used for verifying data and will be destroyed after data collection)	
Yes	No

If you require any further information about this study, please contact Calvin Mole on (021) 406 6026 or by email at calvin.mole@uct.ac.za. AND/OR contact Tyrian Laubscher on 066 267 3797 or by email at lbstyr001@myuct.ac.za.

Thank you for your time and participation. Please sign the consent form below if you wish to participate.

______________________________ ______________
Signature of Participant Authorising Consent Date

____
Printed Name of Participant Authorising Consent

As a representative of this study, I have explained to the participant the purpose, procedures, possible benefits, and possible risks of this research study, and the purpose of the data collection.

______________________________ ______________
Signature of person obtaining consent Date

____
Printed name of person obtaining consent

Page 1 of 1