**POST MORTEM INSECT SUCCESSION AND DEVELOPMENT PATTERNS ON DECOMPOSING PIG (*Sus domesticus* L) CARRION IN ELDORET AND MARIGAT, KENYA**

**BY**

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# DECLARATION

**Declaration by the Student**

This thesis is my original work and has not been presented for a degree in any University. No part of this thesis may be reproduced without the prior written permission of the author and/or University of Eldoret.

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## DEDICATION

This research thesis is dedicated to dad, and mum, siblings and all my close friends who have shared with me all my past and present success and tribulations. To Zoology students aspiring to be future forensic entomologists and all the crime researchers who will use information from this study to improve their future learning experiences in Kenya and beyond, “*Karibuni*”.

# ABSTRACT

The death of human provides a decomposing media in the carcass/carrion which act as reservoir for rapidly changing habitat and food source for microorganisms and arthropods. The pattern of micro/macro-organisms succession on the decomposing carrion could be used as surrogate for determining important foresensic information. This method has not been used in Kenya in medicolegal/forensic investigations. This study determined post mortem insect succession and development patterns on decomposing pig (*Sus domestica L*) carrion in Eldoret and Marigat in Kenya. Decomposition followed all the stages (Fresh F for < 1 day, bloat B for 1-2 days, decay D for 3-8 day, advanced decay AD for 9 to 23 days, and and dry DR for 18 to 36 days). Stages F and B, were concomitant and for both carcasses but the rate of decay at stage AD was higher in Marigat than in Eldoret. Internal temperature at Marigat was significantly ($P < 0.05$) higher than that of Eldoret at all developmental stages, where it initially increased with progression of decay. Insect succession, species richness and abundance was high in Eldoret than Marigat. Insects including Lepidoptera that fed on carrion exudates, Hymenopterans as parasitoids, predatory Dermaptera were observed on the carcass upto advanced decay, while Diptera occurred at the fresh stage and bloat stage and lasted until the advanced decay with coleopterans colonizing the carrion late at decay stage and staying longer until the dry stage, most Hemiptera colonized at the fresh stage and stayed throughout the decay period without any significant change in their abundance. The succession pattern was similar in Eldoret and Marigat but they differed in their residence time. In terms of insect lifecycle: Diptera and Hemiptera laid eggs in fresh or bloat stage while coleopteran laid eggs at decay stage. The eggs hatched and developed to first and second larval instars during decay and progressed to third instars in advanced decay stage and the adult insects emmerged before the onset of the dry stage. However, only coleopterans with enzyme chitinase completed their life cycle in the dry stage. Also, the abundance of insect at bloat, decay due to fluidy status of the carrion, dry stages and advanced decay were positively associated with ambient temperature, soil temperature, relative humidity and wind speed that affects insects landing time for biological activities. Meanwhile, at each stage of decay, the combination of environmental factors that influenced the insect fauna was species specific. The study estbalsih that even in proximate geographic areas, the decomposition and insect succession are affected by the environmental variables.

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# CHAPTER ONE

# INTRODUCTION

## 1.1 Background of the study

Each year, humans die throughout the world either naturally or through unnatural causes including homicides, suicides, and accidents or simply due to unknown circumstances (Costa *et al.*, 2018; Nagel, 2018; Kalish, 2019; Cerezo-Román & Anderson, 2020). In cases of unnatural deaths, human bodies may be recovered by accident, investigation and archeological excavations or through reported cases (Franco *et al.*, 2018; Parveen *et al.*, 2018). The most difficult information to obtain from the corpse, yet very relevant to be ascertained by law is the clues about the cause and time of death (Shapiro-Mendoza *et al.*, 2017; Mitchell & Drake, 2020). Observation, physical as well as chemical test can ascertain the cause of death however, the time that the death occured is always a challenge (Reinhard *et al.*, 2018; Wescott, 2018b; Choi *et al.*, 2019).

After death of animals and human beings, cells stop functioning and autolysis commences when enzyme start digesting the cells and the body starts to decompose (Kim *et al.*, 2018; Almulhim & Menezes, 2019). The soft tissues in the gut are destroyed by bacteria producing cadevaric volatile organic substances imcluding acidic substances, aromatic carbon and hydrocarbons, oxygenated compounds, sulphurus and nitrogenous compounds and in some extreme coondeition they liberate hydrogenated compounds (von Hoermann *et al.*, 2016; Ioan *et al.*, 2017). The process comes to a sudden ceasation during skeletonization of the cadaver. It has been established that a number of animal models or carrions that can resemble the decomposition patterns of human corpses are available including rats (Rigalli & Di

Loreto, 2016; Perkins *et al.*, 2018; Belk *et al.*, 2019), dogs (Fouda *et al.*, 2017; Guerroudj *et al.*, 2017; Zeariya & Kabadaia, 2019), other rodents (Turner *et al.*, 2017; Dautartas *et al.*, 2018; El-Gawad *et al.*, 2019), monkeys (Prins *et al.*, 2017; Sebastião & Prado e Castro, 2018), broiler (Moophayak *et al.*, 2017) and domestic pigs (Koffi *et al.*, 2018; Matuszewski *et al.*, 2019). Among these animal carrions, domestic pigs (*Sus domestica*) is acknowledged as the closest analogues to humans in decomposition characteristics is currently one the most widely used in experiments to surrogate human corpses (Armstrong *et al.*, 2016; Connor *et al.*, 2018; Dautartas *et al.*, 2018).

For along time, the pathology- based indicators in human/animal carrions relied upon body decomposition parameters such as body temperature (Connor *et al.*, 2018; Dautartas *et al.*, 2018; Lutz *et al.*, 2019), rigor mortis (Brooks, 2016; Gelderman *et al.*, 2019), livor mortis (Chen *et al.*, 2015; Brooks, 2016; Forbes & Carter, 2016), microbial change (Crippen *et al.*, 2015) and biochemical conditions of the body (Armstrong *et al.*, 2016; Ioan *et al.*, 2017; Almulhim & Menezes, 2019) which appear early, usually within the first 72 hours (Armstrong *et al.*, 2016). However, these indicators do not provide the required forensic information after the elapse of 72–80 hours (Belk *et al.*, 2019). Therefore other indicators that provide the requisite pathological information relative to decomposition dynamics have been explored.

It has been widely recognized that decomposing human/animal carrions act as an habitat or micro habitat with food resource that allow for invasion  ants, rodents, pests and other microorganisisms including bacteria, fungi, invertebrate and vertebrate scavengers (Pascual *et al.*, 2017; Singh *et al.*, 2018; Barton & Bump, 2019). There are stages of the decomposition process that allows for the creation of favourable mini or microenvironment that can be colonized by the invading species in an orderly sequence (Iancu *et al.*, 2018b; Singh *et al.*, 2018; Heo *et al.*, 2019). This ecological principal has been relied upon to estimate some of the forensic parameters using abroad range of species of arthropods.

The high diversity, population dynamics, reproductive strategies as well as occupation of consumer trophic tiers of arthropod community allow them to play important roles in the ecosystem structure and functions (Iancu *et al.*, 2018b; Idowu *et al.*, 2019). According to the arthropod succession theory on carcasses as proposed by Mégnin in 1894 (Michaud *et al.*, 2015; Ksiazek-Mikenas *et al.*, 2018), there are four categories of arthropods. The first category are the Necrophages which feed and breed on the carrion tissue and include Diptera (e.g., calliphorid flies, sarcophagids, muscids and piophilids) and Coleoptera (e.g., clerid, silphid and dermestid beetles). Secondly are the parasites of necrophagous species where histerid and staphylinid beetles as well as Hymenoptera parasites of the Diptera eggs and larvae dominate. The third category is the omnivorous insect species which nourish from the fauna associated with the carcass/corpse. The fourth category is the incidental/accidental species occurring largely by chance or uses the decaying remains as habitat. Progression of decomposition on the cadaver/corpse allows each stage to turn out to be attractive to other groups of arthropods such as caddeveric insects (Frątczak & Matuszewski, 2016). These have been identified and subsequently utilized as markers for the estimation of the requisite forensic parameters (Sanford, 2017; Olea *et al.*, 2019).

During forensice investigation, the time elapsed between death and discovery of a body denoted as post-mortem interval (PMI) remains the most important (Cai *et al.*, 2018; Haddadi *et al.*, 2019). This is determined using the sequence of presence of different orders and /or species of insects. Insects on corpses provide longevous information on medium- and long-term scale to estimate PMI, since medical measures are not functional (Zar & Huang, 2018; Al-Qahtni *et al.*, 2019; Mahmood *et al.*, 2019). An understanding of the trajectory of insect succession on carcass in a localized biogeographic area allows for the analysis of the length that the corpse was eposed to which may involve a rudimentary understanding of PMI (Dao *et al.*, 2018; Siri *et al.*, 2019). Neverthless, to estimate PMI then more information that entails an understanding of the species and their biilogicaal cyclces from eggs stages should be considered (Açikgöz & Açikgöz, 2018).

Geographic region or biogeoclimatic zone has been highlighted as one of the factors that affect sucession of insect species (Dao *et al.*, 2018; Pérez-Marcos *et al.*, 2018; Von Hoermann *et al.*, 2018). This implies that to understand findings in the insect succession patterns and draw conclusion on important forensic parameters, the region and ambient physical conditions must be considered. This is because species of insects that occur in one particular area are always specific and cannot be used to generalize the findings for another geographical area (De Faria *et al.*, 2018; Lutz *et al.*, 2019). The variation in biogeography on corpse carrion should be considered during forensice investigation as faunal succession are affected by some environemtnal variables such as humidity, locality, temperature, wind patterns and rainfall (De Faria *et al.*, 2018; Koffi *et al.*, 2018; Sebastião & Prado e Castro, 2018). Therefore environmental data remains significant during forensic investigations.

There is great deal of variability in specific insect species, biology as well as life cycle strategies and during colonization of the carrion the differences with stage of decomposition vary markedly for each species relative to the regional geographical conditions making it important to obtain a set of basic information to enable one to evaluate the potential of insects in forensic entomology. The bulk of the available of published works has addressed scenarios in developed countries of Europe, United States, and Australia, but extension of the same in Africa is poor (Villet, 2011; Mona *et al.*, 2019; Munaier, 2019). Within the African region, carrion studies heavily focuse on South Africa and Egypt, with a few studies in Cameroon, Angola, Ghana and Nigeria (Sebastião & Prado e Castro, 2018; Lei *et al.*, 2019; Ojianwuna *et al.*, 2019). Kenya lacks any published studies of entomophauna associated with a decomposing carrion in any animal/human carrion except for an elephant in the wild (Coe, 1978). Consequently, such studies must noww be incorporated into forensic investigations especially in numerous reported cases of homicides, sucides and inexplicable murder frenzy widely reported in Kenya. The current study seeks to generate data on arthropod succession patterns on carrions in Kenya.

### 1.2 Statement of the problems

During death the first key forensic parameters should be the duration of time between and discovery of the dead body or PMI. This is the most important part of evidence for any postmortem report. Death attract variety of insects feed on the body of the decomposing animal which the remains as a resource for various behavioural-ecological activities. Once they have commenced feeding, then insects will proceed to use the decomposing body as an oviposition media. Several other species including predators are also attracted to feed on other arthropods consuming the food on the carrion. It is this pattern of insect succession on the cardaver that can be useful in forensic entomological study (Iancu *et al.*, 2018a; Ramos-Pastrana *et al.*, 2018; Feddern *et al.*, 2019). Each region has unique range of species of insects that must be known in order to understand their succession patterns of insects on the corpse or animal carrion. Yet to the researcher's knowledge, there is no research done to establish arthropods of forensic importance in Kenya and therefore, the association between decomposition of dead bodies and insect colonization has not been investigated in Kenya even at the baseline level. In particular, there is currently lack of information on insect succession pattern on decaying carrion. Lack of any baseline studies makes it difficult to establish the nature of intra - and interspecific interactions and progression of decomposition, which is key in forensic entomology. More importantly, there is no information on the effects of many biotic and abiotic factors, on insect colonization and development patterns. Availability of this knowledge in Kenya, provide additional important evidence that strengthens the understanding of some of the cryptic forensic issues in the country.

## 1.3 Justification of the study

The results of this study will help improve the predictability of foresnice investigations by incoperating measurable ecological parameters to help decipher the phenomena. Hence the results of the current study will improve the theoretical understanding of carrion ecology through empirical studies in a region where such studies are rarely carried out. In the case of carrion ecology, the most pressing areas for research are in understanding of the insect succession process and to be able to predict more accurately on the required forensic evidence when similar situations occur in future at the same location or other locations in the proximity.

A major challenge of forensic entomology is the pronounced geographic variability of the carrion insect community that diminishes the reliability use of the available data from one biogeographic area to another (Castro *et al.*, 2019). Hence, this requires generation of data for each case. Insect species composition can vary greatly at different localities, thus forensically important insects should be enumerated for specific area to help estimate PMI. Such information in forensically important insect species in Kenya is lacking. Results from this study will contribute to enhancement of the database on distribution and diversity of insects of entomological relevance as well as the influence of environmental factors on their ecological distribution.

The baseline information on the important insect species that affect carrions and the changes relative to biogeography is useful to further studies on the influence of biogeographical variation on insect succession on carrions. This will shed light on potential use of the generated data in local forensic science, including estimation of PMI.

## 1.4 Objectives of the study

### 1.4.1 Main objectives

The objective of this study was to determine the post-mortem insect succession and development patterns on decomposing pig carrion in Eldoret and Marigat, Kenya

### 1.4.2 Specific objectives

i. To determine the decomposition patterns and the role of environmental factors during postmortem decomposition of pig carrion in Eldoret and Marigat

ii. To establish succession patterns of different species of insects during postmortem decomposition of pig carrion in Eldoret and Marigat

iii. To determine development patterns during postmortem decomposition of pig carrion in Eldoret and Marigat

iv. To evaluate the role of environmental factors on insect succession patterns during postmortem decomposition of pig carrion in Eldoret and Marigat

### *1.5 Hypotheses*

$Ho_1$: There is no significant difference in decomposition patterns and influence of environmental factors during postmortem decomposition of pig carrion in Eldoret and Marigat

$Ho_2$: There is no significant difference in insect succession pattern during post mortem decomposition of pig carrion in Eldoret and Marigat

$Ho_3$: There is no significant insect development pattern during postmortem decomposition of pig carrion Eldoret and Marigat

$Ho_4$: There is no significant influence of environmental factors in insect succession during postmortem decomposition of pig carrion in Eldoret and Marigat

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Forensic entomology in research

To establish the cause of death in an endaviour to resolve the puzzle of homicides and suicides is not an easy task as it involves efforts of several discipline such as medical personell, crime detectors, technicians, witnesses, pathologists, entomologists and could involve suites of other professionals who are not in the medical field (Burcham & Jordan, 2017). However, in many cases, not all these disciplines are always represented and the situation is dire in developing countries. There are several inexplicable mortalities under investigation which is purely a legal process. The cause of death remains paramount in the investigation which is the prerogative of the a forensic pathologists and the report finally handed over to the physician (Mike Groen & Berger, 2017). The pathologist will most likely conduct all forms of examination of the corpse for antemortem injuries, postmortem changes, and attempt to recover physical evidence for the cause of death (Ribaux & Caneppele, 2017). The manner, time and cause of death are other important paeamters to be determined by the pathologist (Ukpo & Boger, 2018; Morewitz, 2019).

Forensic entomology advances application of insects in legal and criminal investigations. These benefit from collaboration with an entomologist who is able to interpret entomological evidence in cases involving a variety of factors such as food contamination, violation of pest control contracts, infant or elderly neglect, ante mortem infestation, or postmortem colonization of humans and wildlife (Christofis, 2018; Vicks, 2019). Medico-legal forensic entomology involves insect evidence in medical and criminal cases of injury or neglect. Such scenarios has been applied to cases of wrongful death or suspected foul play for decades, with a surge of research in this area in recent years (Krinsky, 2019; Lei *et al.*, 2019).

Insects often discover deceased individuals before humans do and their presence provides useful information to investigators (Parkhideh *et al.*, 2018). Thus the insects become important sampling parameters to provide evidence of forensic nature. Once initial colonization occurs, insects arrive in a predictable pattern according to availability of particular food source/refuse resources, and this predictability allows entomologists to relate the time the carcass has been exposed to the insects that are observed (Mahmood *et al.*, 2019; Mona *et al.*, 2019). In this case, evidence of presence of insects can be as minute as a single egg mass or as prominent as a large maggot mass when a carcass is in the active decay stage. At other times, only the dry remains of the carcass/cadaver are present and the insect evidence is left behind. Insects that feed on dried remains or fly puparia may provide clues as to how long the remains have been there (Cruise *et al.*, 2018a). Most research on the use of insect evidence in human death cases are carried out on domestic pigs, *Sus scrofa domesticus* which is close resembles a human analogue (Connor *et al.*, 2018; Cruise *et al.*, 2018a). Using pigs allows investigators and researchers to manipulate the ante and postmortem events surrounding death to simulate situations that human decedants may have encountered (Martín-Vega *et al.*, 2019). When properly done, this can aid the justice system to extrapolate the time since death based on evidence of insect activity on the human carrion.

Until recently, apllying forensic entomology wanst a common tool in legal investigations even though it has been in practice for centuries (Lei *et al.*, 2019). For many years medical examiners and crime scene investigators would remove and discard insects found on and around bodies (Ghosh *et al.*, 2018). Recently, through the continued investigation of the ecology surrounding death and the reliance of law enforcement on scientific findings, forensic entomology has made great progress as an asset to the justice system. In 2018, the National Research Council published a 752 page review of the forensic sciences and called for more objective research that focuses on standardization within the forensic sciences (Bell *et al.*, 2018). Efforts have been made to comply with the NRC's recommendations to improve the accuracy of field techniques (Roux *et al.*, 2018; Iacono & Ben-Shakhar, 2019). These efforts have focused on more precise terminology and the exploration of potentially confounding factors associated with the precision of estimating the period of insect activity. However, at local levels, it is still difficult to comply with this recommendation due to paucity of research information on forensic entomology.

Entomological evidence can support or contradict the interpretations of other types of physical evidence and witness accounts. Blood feeding insects found on a victim can contain DNA from its original host, which can be used as evidence of the suspect's contact with the victim (Mahmood *et al.*, 2019). The most commonly applied use of insect evidence is through the estimation of $PMI_{Min}$ (Mahmood *et al.*, 2019). Currently, there is a shift from the terminology of the $PMI_{Min}$ to the period of insect activity (PIA) (Vanin, 2018; Wang *et al.*, 2019). The PIA ideally includes the initial discovery of the carcass by the insects, their mating, and oviposition on it, whereas the $PMI_{Min}$ estimates the minimum period of time that insects have utilized the carcass. The PIA can be used in cases of antemortem infestation, while the $PMI_{Min}$ relies on the positive identification of the insects collected and determines the timing of infestation as ante-, peri- or postmortem (Cruise *et al.*, 2018b). In this thesis, $PMI_{Min}$ will be used because of its current acceptance in the forensic entomology literature.

Witness accounts and evidence of the victim's last activities can provide a rough timeline, assuming these sources of evidence are accurate. When insect activity is utilized to construct a timeline of victim exposure, the estimation of a $PMI_{Min}$ based on the insect biology includes a specific confidence interval (Sterzik *et al.*, 2018; Choi *et al.*, 2019). The accounts of witnesses and victims activity may be wholly inaccurate and typically have no measureable confidence interval. An estimate of the $PMI_{Min}$ is generally constructed in response to insect colonization patterns and life cycle on the carcass or decedent. Often the first insects to arrive and lay eggs on a carcass are flies (Order: Diptera). Additionally, because insects colonize carcasses in a predictable pattern, the $PMI_{Min}$ can be based on the composition of insect fauna on the carcass (Wescott, 2018a). The natural ability of insects to locate and utilize decaying material, while having temperature dependent growth, is what makes them valuable as evidence in $PMI_{Min}$ estimation.

Carrion ecology has been well studied, and the community of insects associated with carrion can be divided by the feeding guilds of insects that hold specific niches in the community (Wallace, 2015; Anderson & Wallace, 2019). Groups of insects other than flies utilize carcasses as food sources and either feed directly upon them or upon the maggots that are utilizing the carcass tissues. After an initial colonization, the carcass is no longer a resource for a single group of organisms, but it becomes a small-scale community ecosystem, or microsere (Metcalf *et al.*, 2015; Heo *et al.*, 2019). Various resources in this micro ecosystem become available at different times throughout the various stages of decomposition of a carcass. Insects utilize the bodily fluids, skin, muscle tissue, hair, hooves, and the contents of the digestive tract and locate the carcass when their preferred diet becomes available (Wescott, 2018a).

The expanding literature in the use of insect in criminology is still minimal in when compared to other field of science. A bibliography published in 2018 listed only 749 references on use of insects in forensics from 1834 to 2019. This includes 434 references between 2001 and 2015. A frequently accented disquiet in this area is that there are few studies conducted across biomass and this makes it quite difficult to get meta-data for cross biomass comparison to enable to infer one possible conclusion, rather than scattered and locally grounded studies. Therefore more studies in relevant biomes including those in the tropics will likely to be welcomed in international literature.

## 2.2 Human animal carrion decomposition process

When mammals die, the process of decomposition sets in starting with breakdown of body tissues (Zerbo *et al.*, 2020). The breakdown of soft tissues of the body during putrefaction is aided by saprophytic microorganisms such as bacteria, fungi and protozoa, a process that is now well understood both in the human and animal carrion (Bugelli *et al.*, 2018; Iqbal *et al.*, 2018). The decomposition process results in the production of gases, liquids and simple molecules. Various gases including carbon dioxide ($CO_2$), hydrogen sulphide ($H_2S$), methane ($CH_4$), ammonia ($NH_3$), Sulphur dioxide ($SO_2$) and hydrogen ($H_2$) produced in the process distend the tissues (Nolan *et al.*, 2019). Along with these, are a variety of volatile organic compounds (VOCs) are liberated (Xia *et al.*, 2019). These volatile substances are intermediate products of decomposition while the large biological macromolecules such as proteins, nucleic acids, carbohydrates and lipids are broken down into their building block components. Carbohydrates are broken down mainly to oxygenated compounds (alcohols, aldehydes, ketones, acids, esters and ethers); proteins to nitrogen, phosphorus and sulfur compounds; while nucleic acids produce nitrogen and phosphorus compounds (Chawla & Patel, 2019). Further, lipids break down to hydrocarbons, nitrogen, phosphorus and oxygenated compounds (Almulhim & Menezes, 2019). However, there may be no recognized sequence in production of these volatile organic compounds, this measuring their production during decomposition may not provide much forensic information about human death including time of death and cause (Sutherland & O'Donnell, 2018; Gunn, 2019). Other authors have argued that in some instances, the compounds can be produced at some stages of decomposition with some degree of reproducibility when there are no adverse changes in some ambient conditions (Procopio *et al.*, 2018; Vanin, 2018). Yet the challenge in maintaining ambient conditions uniformly throughout various biomes is untenable.

To establish more relevant information to forensic science, the decomposition process is commonly described in terms of several successive phases or micro- seral stages, usually based on the condition of the carcass, which follow coded timelines (Belk *et al.*, 2019; Hu *et al.*, 2019). These phases are markers for diagnostic purposes indicating time that may have elapsed since death. Some authors distinguish up to eight stages, while others have identified only three, that are subdivide into sub-stages. However, these stages should not be regarded as clearly indefinable moments distinguishing one from the other but rather as a sequence ofdifferent phenomena that overlap leading to uninterrupted progression of decay until the organic matter is completely destroyed (Buekenhout *et al.*, 2018; Ramos-Pastrana *et al.*, 2018). The stages commonly mentioned in carrion studies are fresh, bloated, active decay, advanced decay and remains (Wescott, 2018a; Siri *et al.*, 2019). The fresh stage follows death immediately, and precedes the formation of significant amounts of gas trapped within the body cavity, which is characteristic of the bloated stage. Once these gases have leaked from the body, the carcass is actively decaying and insect colonization has usually occurred (Koffi *et al.*, 2018). In advanced decay, fluids leak from the carrion and the remaining soft tissues desiccate to various degrees. Rain can moisten carrion in the advanced decay stage sufficiently that it returns to the condition of the active decay stage (Bugelli *et al.*, 2018). Vertebrate scavengers can cause the elision of the bloated and active decay stages, substantially depressing the numbers of insects that colonize the body (Cruise *et al.*, 2018b). Remains have little or no soft tissue and consist of bones, teeth and keratinous materials, and may be dispersed by vertebrates. Eventually the remains weather away through the effects of heat (which promotes fracturing and chemical reactions) and moisture (which promotes weathering directly and by encouraging microbes and algae) (Kaur & Bala, 2018; Lutz *et al.*, 2019).

Empirically, the stages grade into one another, and some of the transitions last longer than some of the stages (Gelderman *et al.*, 2019; Keshavarzi *et al.*, 2019). Despite the time sequence, no one stage has been ascribed absolutely to a well defined interval of time since death, due to compounding factors (Wescott, 2018a). However, the putrefactive changes can be used for estimating the PMI only if they are suitably integrated with the environmental and circumstantial elements (Buekenhout *et al.*, 2018). For it to be effective, the transition from fresh to bloated stage depends on how promptly microbial activity within the body generates gas; from bloated to active decay is affected by how soon insects provide vents for the gasses by eating into the entrapping tissues, and is therefore associated with colonization of the internal tissues by maggots (Gelderman *et al.*, 2019). Also the transition from active decay to advanced decay is usually attributed to activities of insects that perforate the body and allow fluids to drain from it (Haddadi *et al.*, 2019). This is anecdotally ascribed to the efflux of mature maggots when they are ready to pupate.

The relative lengths of the bloated and decay stages depend in part on which species are present and in what numbers. For example, in Egypt in spring, the blowfly *Lucilia sericata* dominates the bloated stage in rabbit carrion, but in summer the flesh fly *wehlfahrtia nuba* fills this role (Aly *et al.*, 2017). *Lucilia sericata* colonises in large numbers, and the carcass is soon perforated and passes into advanced decay stages, while *wehlfahrtia nuba* colonises in smaller numbers, leading to slower consumption of the carrion, protracted bloating and a later transition to advanced decay.

Because a mechanistic link is often made between the stages of decay and the activities of specific insects, it is regularly claimed in the forensic literature that each micro-seral stage has its own community (Al-Qahtni *et al.*, 2019). However, empirical qualitative examination of published data from research carried out in different countrie in Africa shows that the match between the stages is erratic and the succession progressive rather than episodic (Tomberlin *et al.*, 2017; Moeti, 2019). Some of this may be attributed to the fact that a typical example of carrion is not a homogenous habitat, since its physical extremities provide microhabitats that may be in different stages of decay to its core biomass (and to one another), thus blurring the putative seral stages. Besides this spatiotemporal heterogeneity, the carrion is never really in a stable state because of the persistent disturbance at the same time scale as the succession process.

Quantitative examination of published studies from outside Africa has shown that the decomposition process is essentially a continuum, and that the apparent stages should rather be treated as milestones that have descriptive value only (Szpila *et al.*, 2015a; Tomberlin & Benbow, 2015). The continuous changes accompanying decomposition increase the temporal resolution, and therefore the forensic value, of succession as a ‘clock’It has been suggested that a different way to describe the decomposition process is in terms of landmark events that are significant to the insects themselves (Szpila *et al.*, 2015b; Singh *et al.*, 2018). A phase of exposure follows death, ending when the body is detected by insects and the period of insect activity starts. This period can be subdivided into phases of detection, acceptance, consumption and dispersal, demarcated by location, colonisation and maturation of the insects, respectively (Amendt, 2018). Similar terminology is used to describe the interactions between parasitoids, or herbivorous insects, and their hosts (Frago, 2016; Abram *et al.*, 2019). Because the durations of these phases will depend on which species is being considered, this description is autecological rather than an account of the decomposition process.

Based on the forgoing and information from extant literature, it is observed that accurate determination of many forensic parameters may be difficult by just looking at decomposition characteristics. This is because; the rate of postmortem decay can be affected by variables of different nature with regard to the carcass itself (intrinsic factors) and the external environment (extrinsic factors). Studying the decomposition of corpse using animal carrion model is well developed for many environments, however it lacks the knowledge on the process would proceed in warm tropical environments that have constant temperature range throughout the day and no drastic varriations through the year. It is also not clear how the available data obtained elsewhere may relate to how changes in the local environmental conditions in Kenya may affect the decomposition dynamics of the human corpse or animal carrion models.

**2.3 Insects succession on animal carrion during post mortem decomposition**

Decomposition of remains of human/animal carrions provide a micro-habitat for the changing physical, chemical and biological environment that may provide shifting food source for a wide variety of organisms ranging from bacteria and fungi to vertebrate scavengers (Crippen *et al.*, 2015; Jordan *et al.*, 2015; Burcham & Jordan, 2017). Arthropods are the major organisms competing for these food resources in most corpse/carrions (Mądra *et al.*, 2015; Mashaly, 2017; Blanar, 2019; El-Gawad *et al.*, 2019). The differences in the total number and abundance of taxa observed depend animal models used and geographical location (Iancu *et al.*, 2018b; Haddadi *et al.*, 2019). The colonization patterns and succession appear related to food source by the insects on the cadaver and form the basis for understanding the succession pattern of these micro-organisms (Lutz *et al.*, 2019). This is ultimately important in explaining important parameters in forensic science including establishing the time of death.

Although insect colonization pattern is understood in many habitats, during the process of corpse/carrion, visitation pattern on the corpse/carrion remains remarkably predictable (Moeti, 2019). However, the dynamics of carrion communities on decomposing corpse is inexplicable based on a stage-based model of decomposition (Palmiere *et al.*, 2019). In several studies, the first and most significant species of insects to arrive at and oviposit on a corpse/carcass are typically primary and then secondary 'wet-phase' imcluding necrophilous flies of the Calliphoridae and Sarcophagidae families (Parkhideh *et al.*, 2018; Keshavarzi *et al.*, 2019). Late arrivals include predators and parasitoids of maggots, such as beetles (Silphidae, Staphylinidae, and Histeridae family) (Mashaly & Al-Mekhlafi, 2016). As the insects and other predators complete the soft tissue of the carrion, organisms adapted to the 'dry-phase' of the carrion progressively dominate until such a point where strict keratophages are left on the corpse (Szpila *et al.*, 2015b). Influx of necrophages often follow rapid process with massive attrition to this community relative to the reduction in carrion food resource (Islam *et al.*, 2016). As the carrion decomposition comes to an end and early invaders have dissapeared, beetles (family Dermestidae), invade (Thakur & Kumari, 2019). Here it is common to note that omnivorous, adventive and incidental species of the necrophages form take a centee stage in the final process (Baz *et al.*, 2015; Dubie & Talley, 2017), conceptualized as an wet to dry carrion ecotone.

Whilst insect succession follows a familiar pattern at the family level, variation are possible at the genus and species levels (Mashaly, 2015; Michaud *et al.*, 2015; Perez *et al.*, 2015) making the overall prediction of PMI more difficult based on a set of earlier studies (Connor *et al.*, 2018). By implication, having more comprehensive data of each region and the prevailing conditions can help in drawing conclusion about succession patterns of insects on cadaver that is of forensic relevance.

Arthropods will contain elements unique to that habitat and components that change within the habitat (Frago, 2016; Dautartas *et al.*, 2018; El-Gawad *et al.*, 2019). The unique components by these arthropods restrict them to geographic ranges and habitat types (Karimi *et al.*, 2018; Kaur & Bala, 2018; Keshavarzi *et al.*, 2019). Taxa diplaying wider geopgraphical distribution may typically be encountered in several habitat types, most being frequently motile for example, flies. When collecting insects from corpse/carcass, some of these taxa even with their unique distribution patterns, will under certain circumstances, prove to be significant in determining the history of the corpse (Rysavy & Goff, 2015). Nevertheless, caution is required when interpreting the insect taxa succession on corpses since not all the insects present have direct role in explicating any meaningful information on forensics, as some insects may appear there accidentally.

Differences in dietary preferences on the cadaver/carcass may also affect species attraction to carrion. However, the nature of compounds produced during decomoposition can also dictate the succession patterns. For example, carrion flies are attracted by sulphur based volatiles organic compounds generated by microbes (Mulieri *et al.*, 2012) which is a common observation in the bloated stage in the temperate region during winter while in summer sulfur- containing compounds do not appear to contribute much towards attraction of flies. Several special purpose insects such as predators and/or parasitoids appear after colonization of the carrion by prey (Silahuddin *et al.*, 2015) based on the useful cues.

The succession pattern can also be explained by competition which remains unclear (DeVault *et al.*, 2011). The species can enhance competition and therefore favour their succession by excreting allelochemicals that are toxic to other species but are harmless to conspecifics (DeVault *et al.*, 2004). For example, blowfly larvae excrete toxic free ammonia, that to exclude establishment of other species in the early stages of decomposition. On the other hand, blowfly maggots develop rapidly by excluding competition and can prevent intraspecific competition by maturing rapidly if needs be, leading to surprisingly low mortality. Many insect are successful colonizers by ameliorating interspecific competition through niche partitioning (Chittoori *et al.*, 2018). Ecologically, this competition is that once the competition behaviour of insects is understood, then it is easier to predict the colonization pattern of the cadaver and this is important in forensic entomology.

Since an understanding of forensic entomology is usually interested in retrodicting succession process of a specific case, so relevant conditions are more or less known and constrained. Through surveying, many different climates, locales, and host carcasses have been studied (Barton & Bump, 2019). From these observations, basic ecological trends have emerged in the understanding of the ecology of carrion. Therefore the succession pattern of insects in this realm is not possible. Considering that insects are bioregion specific, research in Kenya is necessary before establishing reliable insect succession patterns on animal carrions.

## 2.4 Insects developmental stages on animal carrion during post mortem decomposition

Many groups of insects including those of forensic relevance have a simple development pattern widely recognized across many geographical areas (Sharanowski *et al.*, 2008). The lifecycle strategies of arthropod taxa are therefore important in forensic situations since relating the development pattern of arthropods and the time it takes to complete this process on the corpse can be an important indicator and subsequently estimator of the time of death (Joy *et al.*, 2006). The development of insects on the decomposing corpse can often indicate a specific stage of succession based on the known life history strateging of many insects. Many researchers have argued that this can be more robust when combined with appropriate weather data, to provide a long term $PMI_{Min}$ (Bell *et al.*, 2018). Surprisingly, the use of lifecycle assessment of the insects on the corpse/carcass and its use in estimating the PMI have been conducted in very few cases (Benecke, 2005; Sukontason *et al*., 2007; Goff, 2009). Nevertheless, precaution is required because dietary differences between species at various developmental stages (which may facilitate morbidity), problem of species identification, sampling these insects at various developmental stages may affect the outcome of such initiatives for forensic studies (Theiler and Downs, 2016).

It has been argued that insect species whose development patterns involved aggressive feeding along the decomposition pathway will develop faster and may eventually act as potential predators on the development stages of other insects. This would make it difficult to find and accurately identify the development stages of other insects on the corpse (Amendt *et al*., 2004). The larvae of this beetle feed on the eggs and maggots of other arthropods, which mean that if there are more than one species in the maggot stage, their development stages may be obscured by interspecific predation. Also the adults of *Trox sp.* have been observed to feed on the pupal stages of many insect species and therefore may interfere with the development patterns of several insect species (Masan, 2013). This represents the classical ‘exterminator’ mechanism of ecological succession in insects may therefore make interpretation of the life cycle response of insects on corpse/carcass rather complex. Nevertheless it is important to understand the role at each life cycle stage.

In many insect taxa, eggs are the first stage of the life cycle. Eggs are laid in batches such as those of sacorphagous species, or in masses such as those of Calliphoridae or beetles in sites that are warm, sheltered, with sufficient food and moisture (de Carvalho, 2009). Because there is development of high temperatures in corpdses/carcasses during early decomposition (Goff, 2009), this may explain the reason why many insects prefer to lay eggs on the corpse. The number of eggs may vary between different species including as few as 20 eggs in some species of hemipteran. Some species may oviposit up to 200 eggs while others such as Calliphora may lay up to 3000 eggs (Anderson, 2011). The main challenge when sampling is on eggs is the inability to accurately identify which eggs belong to which species. This is an area that requires an experienced taxonomist, yet in developing countries like Kenya, there are many instances where identification of eggs of different insect species is not easy to accomplish due to inadequatcy of this expertise. Nevertheless in many investigations, eggs are identified by the chorion which is often spotty and reticulate though use of scanning electron microscopes. This is also achallenge since not many institutions in Kenya can afford to have electron microscope.

For many holometabolous insectspecies of forensic significance, the eggs hatch into first instar larvae, which initially feed on fluid exuded from the body and then migrates into the body. The emergency of this stage will indicate the time the adult insects arrived on the corpse. For instance, many species of Calliphoridae first instar larvae will hatch from the eggs within a day while that of Sarcophagidae may take longer, up to 36 hours (Greenberg and Szyska, 2014). Thus the time the insects arrived on the corpse can be estimated if the larvae are sampled and identified accurately. The duration between first instars and second instar larvae also vary greatly between different species and it takes about 48 hours in blowflies and 45 hours in some species of flesh fly. In some beetle species, the duration may be up to 56 hours (Wright and Eckert, 2007; Midgel *et al*., 2012). Once the larvae moults into third instar stage, the bigger size maggots are distinct but occur in masses. At this stage, these maggots migrate and seek a place to pupate and therefore this stage is not very useful for many entomologists doing forensic studies. This is because some of the few studies available have established the pupating maggots move to cooler areas, mainly away from the corpse. In earlier study, Greenberg and Tantawi (1993) established that most of the third instar larvae of blowflies were not on the animal carrion.

After the larval stages, the next stage are the pupae. The pupa stage is a dormant stage during which larval cells and tissues are re organized to form the adult insect. The pupa does not move or feed. It is easy to distinguish the pupa on a corpse because they use elaborate spiracles in the anterior for breathing (Dhadialla *et al*., 2009). In blowfly the stage between pre-pupa to pupa takes 4 days.

There are also very few studies on the pupal stages on the corpse/carcass and therefore, making meaningful interpretations remains a big challenge in forensic science. The pupa stage resides within a puparium and in most cases, will be found in suitable warm or moist places within the corpse. Pupae undergo transformation from larval body form to adult fly without feeding and therefore corpses are not one of the suitable pupation sites. Only one study is currently available on the use of pupae for estimating PMI (Richardson *et al*., 2013). The duration between pupation and emergence of adult insects takes 10 days in some blowfly species and therefore this may provide a basis for estimating the time of adult insect arrival on the corpse.

The development between pupae to adult fly takes between two to four weeks depending on insect species, with large variations reported between sarcophagus and Calliphora as well as in beetles (Singh and Sharma, 2008). In the natural environment, the pupae change colour to reddish brown and dark mahogany brown or black depending on the species, while becoming oval-shaped object resembling a cigar corpse. This change in colouration may impair correct identification of pupae of one species from the other and makes forensic studies more unpredictable. Nevertheless, forensic studies on the changes of pupa to adult parameters on corpses/carcasses are rare, which does not allow any meaningful conclusions to be made on PMI using emergence of adult insects from pupae. Once adults emerge from pupae, they also lay eggs on the decaying corpse/carcass. Female blowflies emerge from pupae and take only 2 days to start laying eggs while flesh flies take 3 days and the entire cycle of the second generation starts again (Theiler and Downs, 2016). There also lies the challenge because one stage can overlap with another similar stage in another generation, especially if the development cycle is not complete, thus making it extremely difficult to determine the important temporal forensic parameters. While considerable emphasis in literature has been given to application of insect succession data to forensic problems, the application of life cycle assessment is complex and has remained poorly understood, and therefore is rarely used in forensic investigations.

### 2.6 Role of environmental factors in insect succession during postmortem decomposition

The rate of postmortem decay and subsequent colonization of the body by insects can be affected by variables of different nature concerning the corpse itself (intrinsic factors) and external environment (extrinsic factors). Variation in the colonization and succession patterns of different species of insects on carrion has been ascribed to variation in the climatic conditions in several studies (Villalpando *et al*., 2009; Battán Horenstein *et al*., 2010; Chelsky *et al*., 2015; Oliveira *et al*., 2016; Sarthak *et al*., 2016; Ahmed and Joseph, 2017; Fusco *et al*., 2017). Changes in the climatic factors that have been shown to affect insect succession by virtue of affecting carrion decomposition include mainly temperature (Battán Horenstein *et al*., 2010; Vasconcelos *et al*., 2013; Matuszewski and Mądra, 2015), precipitation (Archer, 2004; Mahat *et al*., 2009), relative humidity (Frederickx *et al*., 2012; Barton *et al*., 2017), solar radiation (Mashaly, 2017; Shaalan *et al*., 2017), and wind (Turchetto and Vanin, 2004; Oliveira *et al*., 2016) among other factors. The role of each of these factors needs to be understood to be able to decipher the insect succession in many decomposition scenarios. Interestingly there is currently no available record of a study in Kenya that has investigated the role of environmental factors on the decomposition and succession of insects in carrions. Therefore such studies are not only necessary at the local level but will also benefit the international entomologists on how climatic factors in the equatorial region where temperature variability throughout the year is fairly constant, may influence forensic entomology.

Temperature is the major factor affecting decomposition (Vasconcelos *et al*., 2013) by affecting the reaction rates and development of bacteria. In general, the reaction rate of chemical processes increases with an increase in temperature (Simmons *et al*., 2010), while temperatures ranging between 25 and 35°C are optimal for the development of bacteria. Providing cellular enzymes remain intact, cell metabolism can be slowed or accelerated by influencing the enzyme systems that control reactions through temperature change (Carter and Tibbett, 2008). The hydrolytic enzymes responsible for the chemical degradation of cellular constituents operate more efficiently at relatively higher ambient temperatures. The microorganisms responsible for the chemical breakdown of tissue are, also, greatly affected by temperature (Campobasso *et al*., 2001a). In particular low or high temperatures will inhibit microbial proliferation thus delaying tissue breakdown and insect colonization (Campobasso *et al*., 2001b). Generally, the rate of decomposition and colonization generally displays a direct relationship with temperature. In the case of the flies, these differences in seasonal occurrence are reflected in their thermo-physiological tolerances (Villet *et al*., 2011). Blow flies are typically heliotrophic and are active and oviposit only in the daylight, from sunrise to sunset (Nuorteva, 2009). Unfavorable temperature either lower or higher than optimal can easily destroy eggs. In some cases, eggs slow down or stop embryogenesis showing a state of quiescence called diapause; however embrogenesis can resume normally once the micro environmental conditions become favourable. Diapause can be conditioned by right ambient temperature. For instance, Nielsen and Nielsen (2015) demonstrated that eggs of several Diptera did not hatch at temperatures below 4°C but at 6–7°C, hatching and larval development occurred. Based on several experimental cultures, Deonier (2008) identified the minimum temperature inhibiting growth of several species of insects in the temperate region ranging from 2.5 to 12°C. However, few such studies have been conducted in the tropical environment where temperature variations are not very large as in the temperate region.

Precipitation or rainfall introduces moisture or water into the environment, which is essential for the biochemical reactions and responsible for tissue degradation (Tabor *et al*., 1997). Hydrolysis is a chemical reaction in which molecules are broken down to simpler forms by reacting with water and affect the decomposition of the carrion and subsequent succession pattern of insects on corpse (Archer, 2014). Hydrolytic enzymes released into the cytoplasm during autolysis degrade molecules through hydrolytic reactions (Grassberger and Frank, 2014). It prevents the soil below the decomposing remains as well as the remains from drying. Moreover, a moist environment encourages the growth of microorganismsincluding those that are responsible for tissue degradation while re-hydrating dried remains allowing for continued tissue degradation (Archer, 2014). Previous studies have established that variation in rainfall patterns affect carrion decomposition rates and subsequently the number of days to complete decomposition (De Carvalho and Linhares, 2001; Kočárek, 2003; Shi *et al*., 2009). In another study, Kyerematen *et al*. (2013) established that pig carrion completely decomposed in 16 and 24 days for the dry and wet seasons respectively, in the greater Accra begin of Ghana.

The rate at which water is removed from the skin surface of a corpse / carcass is an important determinant of the effect on decomposition of underlying tissues as well as the skin (Aturaliya and Lukasewycz, 1999). Excessive evaporation of water from the skin surface into the surrounding environment may lead to the mummification of underlying tissues. In arid climates or in circumstances of extremely low humidity dehydration of the skin occurs rapidly producing a hard and leathery covering, impermeable to the transfer of water from underlying tissues to the surface (Goff, 2009). The mummified skin covering retains moisture within the visceral tissues thereby providing an environment for continued tissue degradation (Aturaliya and Lukasewycz, 1999). Mummification has a preservative effect and mummified tissue can remain intact for several years (Mann *et al*., 2011).

Data in one area cannot be applicable to another area because, the process is driven by several external or environmental variables (Campobasso *et al*., 2001; Slone and Gruner, 2007; Singh *et al*., 2016; Chelsky *et al*., 2016). Therefore, differences in the outcomes from one region to another may implies that the environmental differences played a major role and therefore, research in region-specific surveys may be one of the ways to account for these impacts of environmental differences from causing variations. The main and most significant environmental factor that has be determined to influence carrion decomposition stems from changes in the prevailing weather or climatic factor (Archer, 2004; Mashaly and Al-Mekhlafi, 2016; Cockle and Bell, 2017a or b). In particular, insects in general are very sensitive to changes in ambient temperature since these affect their growth and development and their daily activities in the environment. The complexity of the interactions amid the various factors is not well known as the effects of each are most often described and researched independently from the other factors. However, it is the influence of climate factors on insect succession that remains more important in understanding the factors that may affect the use of insects in forensic entomology. Although studies of the individual climatic factor on decomposition and subsequently, insect colonization patterns on carrion is now well known, the combined effects of these environmental factors is rarely established in most research. Perhaps this stems from lack of more powerful and robust methods of analysis using multivariate analysis (e.g., Voss *et al*., 2009; Barrios and Wolff, 2011; Michaud and Moreau, 2013).

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 Description of the study sites

Studies were conducted at the University of Eldoret within Uasin Gishu County and Marigat area within Baringo County (Figure 3.1). These two study sites were located about 198 km from each other. The first site was located at the University of Eldoret in Uasin Gishu County which is within the vast Rift Valley Province of Kenya. Administratively, Eldoret is the head quarter of Uasin Gishu County comprising of five administrative constituencies namely Moiben, Kapseret, Soy, Kesses and Ainabkoi. It lies at an average altitude of 2100 meters in the high altitude area. Uasin Gishu has grown over time from a small centre to a large urban area with population of 900,000 people (Kenya National Bureau of Statistics, 2010). According to the Meteorological department of Kenya Eldoret is regarded as a climatically favorable town receiving adequate rainfall each year averaging to 1200 to 1600 mm. Long rains begin in March and end in June. Short rains begin in August and end in September. However due to global warming weather patterns have been shifting slightly over the years. Temperatures range from mean maximum of 30°C and a mean minimum of 22°C (Meteorological department, Uasin Gishu County). The soil is fertile and moderately humid.

The second site, Marigat covers an area of 177.5 $km^2$ and is situated in Marigat division (Latitude 0°13’ N and 1°40’ N; Longitude 35°36’ and 36°30’ E) in Baringo County (Latitude 0°12’ and 1°36’N and longitudes 35°36’ and 36°30’ E). Marigat is an arid and semi-arid (ASAL) region in the country with the average annual temperature and rainfall of 24.6°C and 671 mm respectively hence dry and hot most time of the year. There are two rainfall seasons with long rainy season starting March to July and short rains from September to November. The altitude ranges from 1200 m to 2600 m asl. The study site is located in Kalro Perkera.

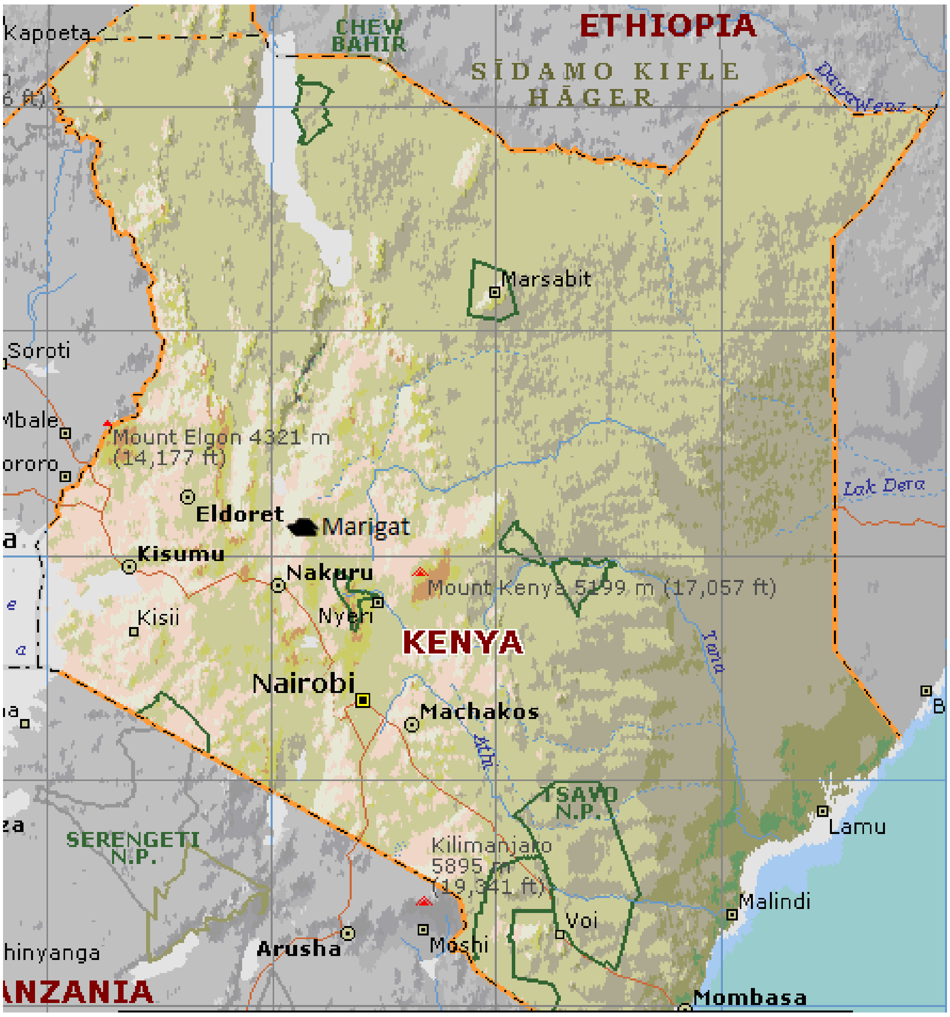



**Figure 3.1: Map of Kenya showing the location of the study sites**

### 3.2 Study duration

The study was conducted between January and March 2017 at both sites. The experiments were carried out concurrently and observations began early in the morning (0600 hrs local time). 3rd January - 23rd March, 2017 implies 79 days.

Observations at the sites were carried on for 26 to 36 days with one visit per day in the afternoon.

## 3.3 Carcass

Two domestic female pigs (*Sus domestica*) carcasses, one weighing 29.7 kg (University of Eldoret) and the other 31.1 kg (Marigat) were used to surrogate human models. They were obtained from the local pork slaughterhouse at exactly 0600 hrs after non-blood shading death. After killing, the carcasses were double - bagged to prevent any unwanted insect activities. Routine monitoring of the carcass for any internal pests or disease was done by the county veterinary department before purchasing the carcass for the experiment. The carcasses were carefully examined for any sign of fly oviposition before they were placed separately inside metal test cages.

## 3.4 Field procedure

The field procedure used standard procedures developed earlier (Rysavy & Goff, 2015). Two to three hours elapsed between death and transfer of the carcass into a cage. A carcass was placed in an enclosed metal cage in such away that the legs protruded towards a hinged door (Figure 3.2). Each of the carrion corpse cage measured 4 m in length × 4 m (width) × 2 m (height) made from metal frame of steel welded tubings. All the sides of the cage was covered with a complete wire mesh (1.27 cm). The carcass was allowed to be in contact with the soil by leaving open the bottoms of the.

### 3.5 Examination of the carcass decomposition and measuring of environmental parameters

To characterize decomposition parameters, the cardaver was checked daily after placement for 2 weeks, followed by once a week until the end of the experiment. At any time of visit, internal temperature was taken using data logger. The progression of decomposition was done through photograph.

At each site the meteorological department provided equipment for measuring ambient temperature, relative humidity and wind speed throughout the experimental period. Temperature was measured using a hand thermometer (3D Model, Brannan & Sons, UK). Relative humidity was measured using hygrometer (HTC Model, UK). For estimation of cloud cover, the eye/manual approach was used. The observer assesses the sky and reports the actual cloud observed as opposed to satellite observation that captures moisture that is not yet condensed into a physical cloud picks moisture 75% of RH catured by satelight as a cloud of the images taken during the study and was measured in oktas=octagon, i.e 8 oktas=whole sky is covered with cloud, if sky is half way covered with cloud is 4 oktas which was observed in Eldoret. Marigat had clear clear. Meanwhile wind speed was measured using an anemometer that recorded knots (2 m/sec) (Local model, Kenya)

### 3.6 Insect collection and identification

Insect colonizing pig carrion were collected using pitfall traps (15 cm deep and 8 cm diameter) and yellow traps (9 cm in height and 27 cm diameter), where 50% of ethylene glycol had been added. Two pitfall traps were placed, with open top flush with the ground surface under the ventral surface of the carcass face. Any insect that was trapped were removed daily and preserved in 70% ethanol. Eggs, larvae/maggots are fly larvae that do not have prolegs unlike in other orders e.g. Coleoptera or Lepidoptera, were sampled simultaneously. Random samples were taken on the first day, 2nd day, 10th day, 15th day and 30th day and identified at the Invertebrate Zoology Laboratory at the National Museums of Kenya, Nairobi using several taxonomic keys and illustrations.

### 3.7 Rearing of Maggots

Occasionally eggs collected were reared. Eggs were collected using a fine brush and metallic spatula and put into vials. Rearing of the eggs and maggots followed standard protocols at room temperature. Eggs were put on fresh pig liver on which the hatched maggots fed until pupation and emergency of adult flies.

### 3.8 Statistical procedures

The frequency and abudance of arthropod species and families that were collected from the traps placed under the pig carcasses in the two regions was compared using a one-way ANOVA. All statistical analyses were performed with a version of 6.0 (Weiß, 2007)or Statistical Package for Social Sciences statistical packages Version 23. (Green & Salkind, 2016). Normality and homoscedasticity of data distribution was checked by considering the skewness and kurtosis (Psaradakis & Vávra, 2018). In case where data was found not to follow the normal distribution (heteroscedastic), log (x+1) transformation was used to normalize all the biological data prior to subjecting to statistical analysis (Dingman *et al.*, 2018).

Principal component analysis ordination determined spatial patterns in insect abundance relative to environmental variables. For each data set, the dissimilarity measure using Bray-Curtis was used. Varimax was applied to rotate the ordination was as to achieve simplified interpretation. Principal Axis Correlation was tested using a Monte-Carlo procedure based on 1000 randomizations.

# CHAPTER FOUR

# RESULTS

## 4.1 Gross carcass decomposition

Carcase decomposition dynamics are shown in Figure 4.1. The fresh stage, no dour, but algor mortis and rigor mortis were observed. In bloated stage, there was detectable bloating starting from the abdomen, livor mortis, discolouration, maggot started to develop inside the body openings and emanation of moderate odour. In active decay face there was pronounced release of gasses, maggot infestation inside the body, dcay oduor, observable putrefaction, deflation and separation of the skin as well as first pupae nears end of stage. At the advanced decay (AD) therefore was defoliation of flesh at the various parts of the body (head, anus, limbs), odour from the internal organs, tissue dehydration and debonification. At the last dry stage (DR), there was little odour, skeletonized tissue, exposed bones and remannats of tissue remnants hich were whitish in colour.

The decomposition took aperiod of 26 to 36 days. Figure 4.2 shows the total number of days for the pig to decompose and complete each stage. Decomposition stage Fresh and Bloat lasted one day each. The carcass decomposition was concomitant in stage F, B and D where they entered each of these stages at approximately the same time. However, the rate of decay at stages AD were significantly ($\chi^2$ = 14.5343, P = 0.0002) higher in Marigat than in Eldoret, where those in Marigat occurred 2 days earlier than Eldoret. The number of days to reach stage DR was significantly ($\chi^2$ = 14.5343, P = 0.0002) different between Eldoret and Marigat. Stage DR in Marigat occurred on day 18 and lasted upto day 26 while in experiment conducted at Eldoret, the stage peaked at day 23 till day 36.


**Figure 4.1: Photographic representation of the chronological decomposition stages observed on pig carcass at Eldoret and Marigat.**

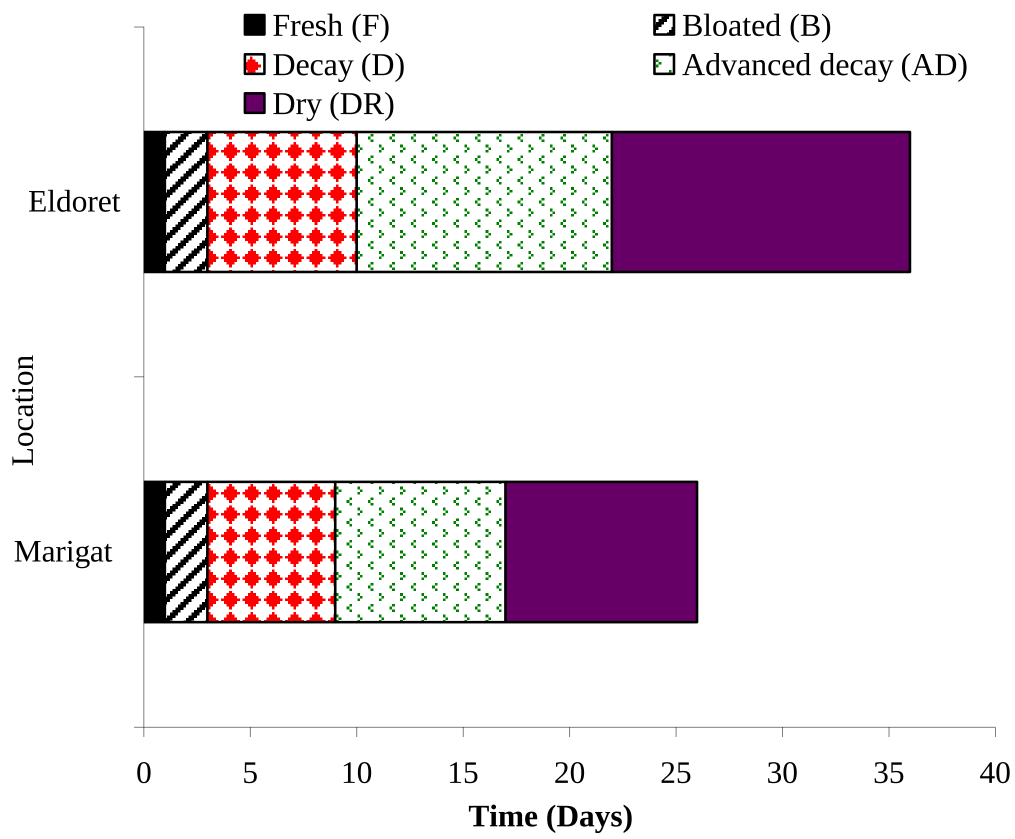



**Figure 4.2: Duration (days) of carrion decomposition in Eldoret and Marigat.**

Data on the internal temperature of the carcass in Eldoret and Marigat during the progressive stages of decomposition is shown in Figure 4.3. Internal temperature at Marigat was significantly ($P$ < 0.05) higher than that of the carcass at Eldoret at all stages. In both experiments, the internal temperatures of the carcasses increased at the beginning of the experiment and continued to increase with progression of decay to a maximum value at advanced decay stage before finally dropping during the progression of dry stage. Internal temperature supports growth of microorganisms by controlling the decomposition rates of carrion.

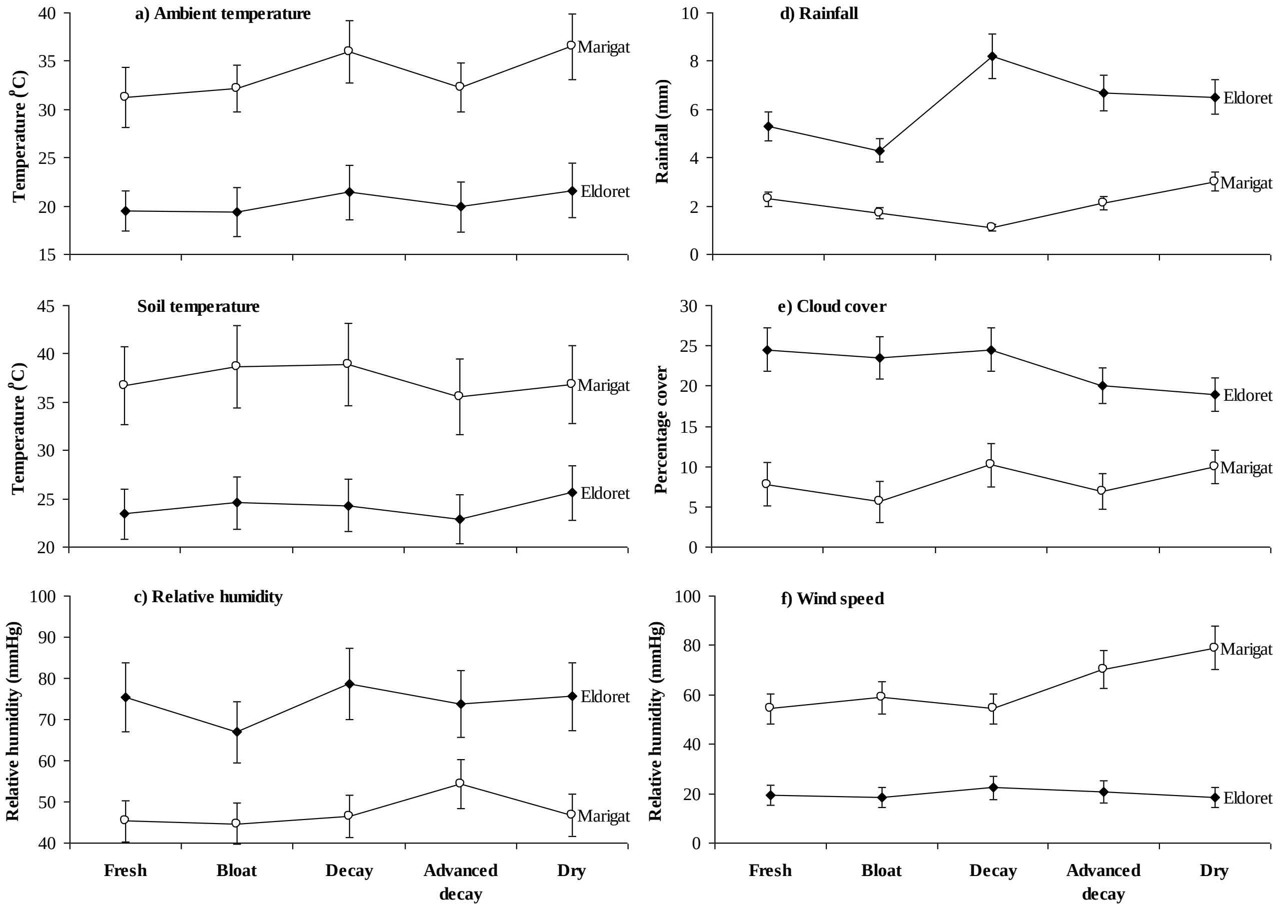



**Figure 4.3a: Data on the ambient temperature measured in Eldoret and Marigat during the experimental period**

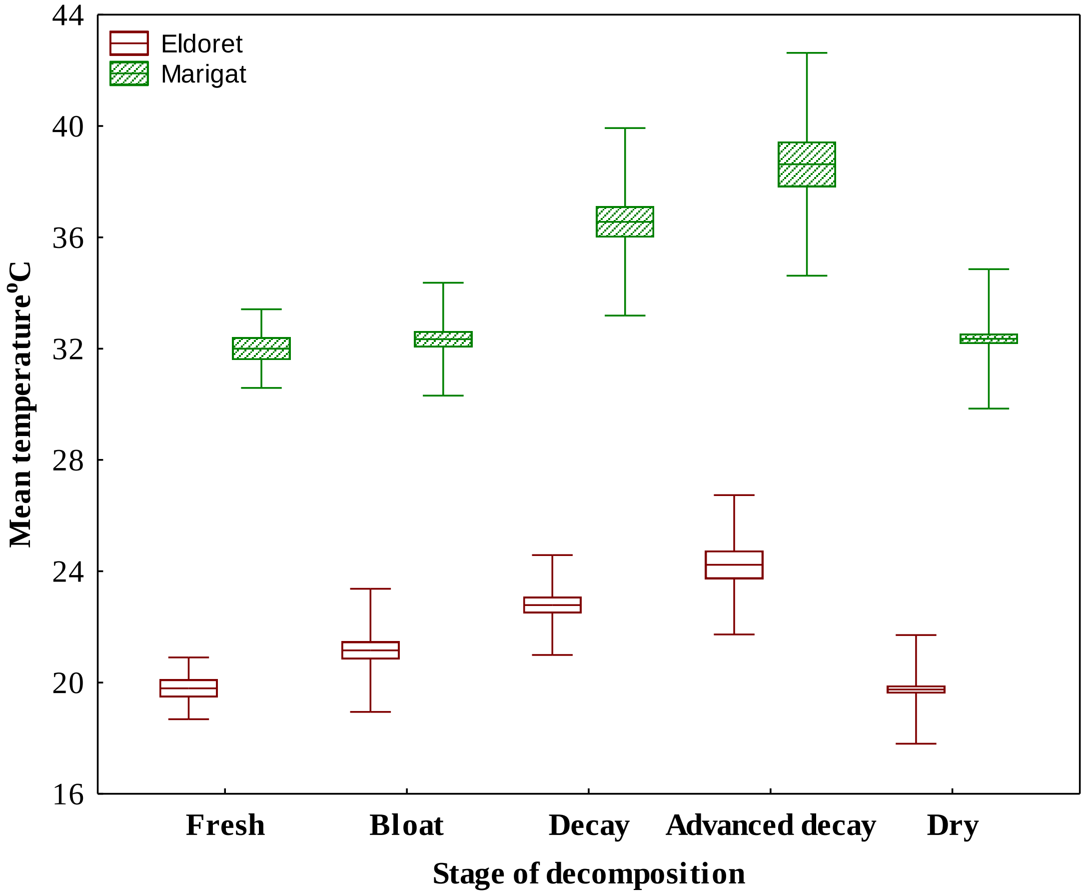



**Figure 4.3b: Data on the mean internal temperature of each carcass at each stage of decomposition in Eldoret and Marigat during the study period**

## 4.2 Insect succession patterns during postmortem decomposition of pig carrion in Eldoret

The insect species composition in terms of absence/presence in the sampling locations at Eldoret and Marigat is provided in Table 4.1. Insects species richness was found to be significantly ($\chi^2$ = 14.5532, df = 1, P = 0.0003) higher at Eldoret with 18 species compared those collected at Marigat site with only 8 species. *Chrysomya albiceps*, *Chrysomya chloropyga*, *Musca dometica*, *Chrysomya regalis*, *Sarcophaga aethiopica*, *Saprinus bicolor*, *Damarsila lethalis* and *Zophosis punctatafasciata* appeared at all the sampling sites in Eldoret and Marigat.

**Table 4.1: Insect species composition in terms of presence (x) / absence (-) in Eldoret and Marigat during the sampling period**

| Order | Family | Species | Eldoret | Marigat |
|---|---|---|---|---|
| Lepidopetera | | *V. cardui* | x | - |
| Hymenoptera | | *Apis mellifera* | x | - |
| Dermaptera | | *F. rehni* | x | - |
| | | *L. riparia* | x | - |
| Diptera | | *S. senegalensis* | x | - |
| | | *C. albiceps* | x | X |
| | | *C. chloropyga* | x | X |
| | | *S. cruentata* | x | - |
| | | *L. cuprina* | x | - |
| | | *M. domestica* | x | X |
| | | *G. maculata* | x | - |
| | | *C. regalis* | x | X |
| | | *S. aethiopica* | - | X |
| Coleoptera | | *S. bicolor* | x | X |
| | | *D. lethalis* | x | X |
| | | *Z. punctatafasciata* | x | X |
| | | *G. simplex* | x | - |
| | | *D. vilpinus* | x | X |
| | | *L. villosa* | x | - |
| Hemiptera | | *A. schulzi* | x | X |
| **Total frequency (%)** | | | **94.7** | **42.1** |

Abundance of different types of insect sampled in Eldoret and Marigat at each decomposition stages are presented in Tales 4.2 and 4.3 respectively. There was significantly higher abundance of insects of all species in Eldoret (405 insects) than Marigat (139 insects). In Eldoret, highest numbers of insects occurred at decay stage followed by advanced decay with dry stage having the least number of insects. On the other hand, in Marigat there were higher numbers of insects in decay stage followed by those during bloat stage and then lower numbers during advanced decay. Species in Order Diptera were dorminant in both sampling areas.

**Table 4.2: Abdudance of different species of insects at Eldoret site during the five decomposition stages over the study period**.

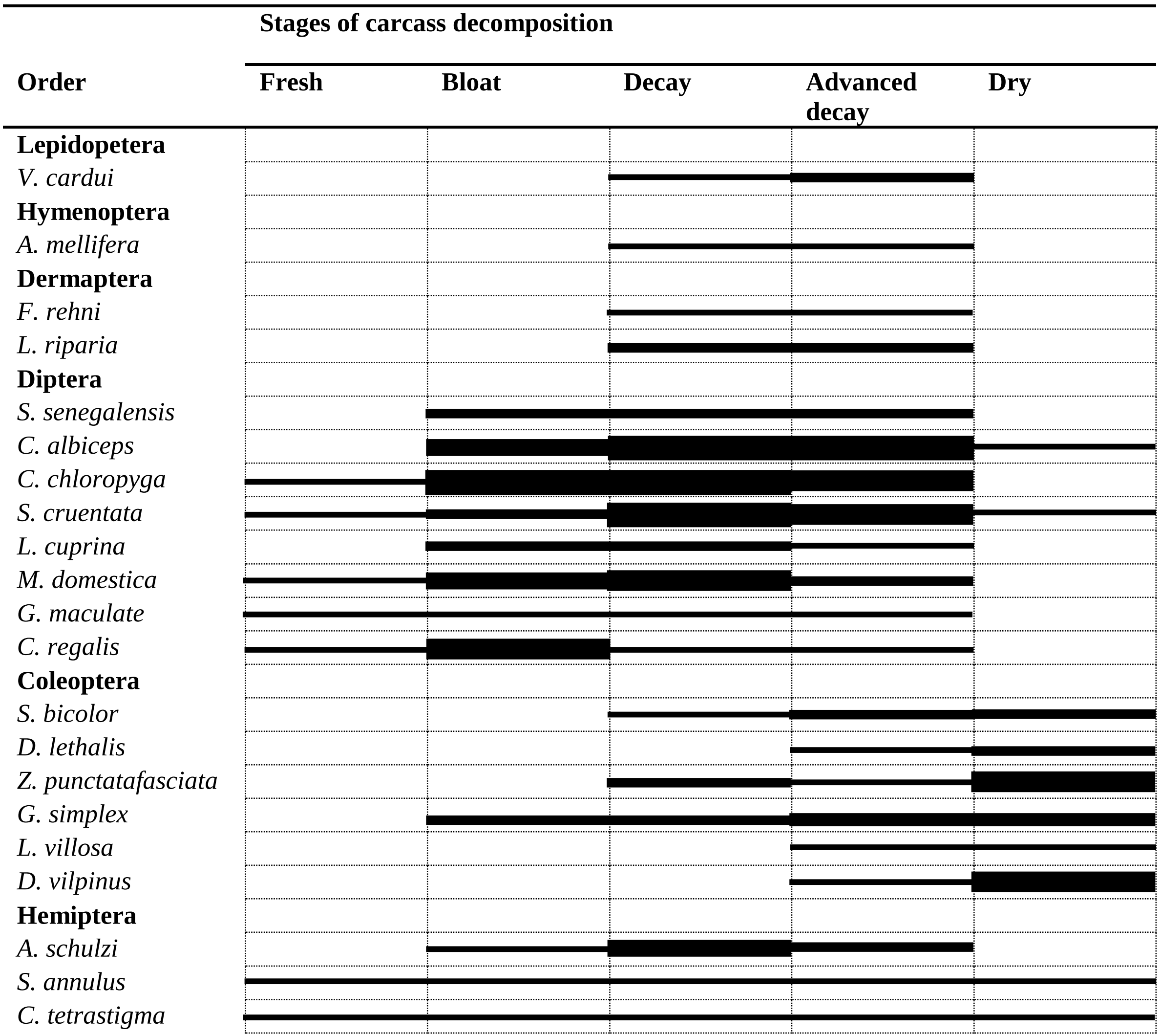
| Order | Species | Fresh | Bloat | Decay | Advanced decay | Dry |
|---|---|---|---|---|---|---|
| Lepidopetera | *V. cardui* | 0 | 0 | 2 | 3 | 0 |
| Hymnoptera | *A. mellifera* | 0 | 0 | 1 | 2 | 0 |
| Demaptera | *F. rehni* | 0 | 0 | 1 | 2 | 0 |
| | *L. riparia* | 0 | 0 | 3 | 4 | 0 |
| Diptera | *S. senegalensis* | 0 | 3 | 3 | 5 | 0 |
| | *C. albiceps* | 0 | 9 | 46 | 34 | 1 |
| | *C. chloropyga* | 2 | 27 | 55 | 19 | 0 |
| | *S. cruentata* | 1 | 4 | 34 | 12 | 1 |
| | *L. cuprina* | 0 | 5 | 3 | 2 | 0 |
| | *M. domestica* | 1 | 10 | 12 | 5 | 0 |
| | *G. maculata* | 1 | 2 | 1 | 1 | 0 |
| | *C. regalis* | 1 | 12 | 2 | 2 | 0 |
| | *S. aethiopica* | 0 | 0 | 4 | 3 | 0 |
| Coleptera | *S. bicolor* | 0 | 0 | 2 | 5 | 5 |
| | *D. lethalis* | 0 | 0 | 0 | 2 | 4 |
| | *Z. punctatafasciata* | 0 | 0 | 5 | 2 | 0 |
| | *G. simplex* | 0 | 5 | 4 | 8 | 11 |
| | *L. villosa* | 0 | 0 | 0 | 2 | 2 |
| | *D. vilpinus* | 0 | 0 | 0 | 2 | 12 |
| Hemiptera | *A. schulzi* | 0 | 1 | 6 | 3 | 0 |
| | *S. annulus* | 1 | 1 | 2 | 2 | 1 |
| | *C. tetrastigma* | 2 | 1 | 2 | 1 | 1 |
| **Total Abundance** | | **3** | **80** | **188** | **121** | **36** |

**Table 4.3: Abdudance of different species of insects at Marigat during the five decomposition stages over the study period**.

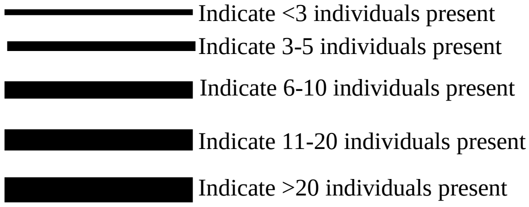
| Order | Species | Fresh | Bloat | Decay | Advanced decay | Dry |
|---|---|---|---|---|---|---|
| Diptera | *C. albiceps* | 2 | 13 | 21 | 11 | 0 |
| | *C. chloropyga* | 0 | 5 | 8 | 4 | 0 |
| | *M. domestica* | 1 | 5 | 7 | 2 | 0 |
| | *C. regalis* | 0 | 3 | 6 | 1 | 0 |
| | *S. aethiopica* | 0 | 4 | 3 | 5 | 0 |
| Coleoptera | *S. bicolor* | 0 | 3 | 5 | 1 | 0 |
| | *D. lethalis* | 0 | 0 | 1 | 3 | 8 |
| | *Z. punctatafasciata* | 1 | 6 | 15 | 3 | 5 |
| Hemiptera | *A. schulzi* | 0 | 0 | 1 | 2 | 4 |
| **Total Abundance** | | **4** | **39** | **67** | **32** | **17** |

Insect succession pattern on carcass at Eldoret site during the study period is shown in Table 4.4. These were from various orders with Lepidoptera presented by *V. cardui*, Hymenoptera presented by *A. mellifera* and Dermaptera presented by *F. rehni* and *L. riparia* displayed late occurrence on the carrion often 3 to 4 days after death in the decay and advanced decay stage and later disappeared from the carcass as dry stage sets in. Two species of dipterans (*S. senegalensis, L. cuprina*) appeared on the carrion after 48 hours at the bloat and stayed until the end of decay while *C. albiceps*, appeared at the bloat and maintained until the dry state although the numbers increased between decay and advanced decay stage. Other dipterans such as *C. chloropyga*, *M. domestica*, *G. maculata* and *C. regalis* displayed similar succession patterns by appearing immediately after setting the fresh carrion at low numbers and increased during the latter stages of bloat to advanced decay and then disappeared from carrion. The only dipteran that occurred in the entire five stages was *S. cruentata.* This species occurred in low numbers at the fresh stage and increased in abundance between decay and advanced decay and then the numbers of flies declined during the dry stage. For the Coleoptera, *G. simplex* occurred throughout the fives stages with increased abundance between bloat and decay stages, while *Z. punctatafasciata* and S. bicolor displayed similar patterns of colonizing the carrion at bloat phase until the advanced decay stage and were higher in abundance at decay stage. Species of Hemiptera represented *A. schulzi* started colonizing the carrion at bloat stage, and at decay stage were more abundant which reduced at advanced decay stage.

**Table 4.4: Insect species succession in each decomposition stage of pig carcass at Eldoret site**

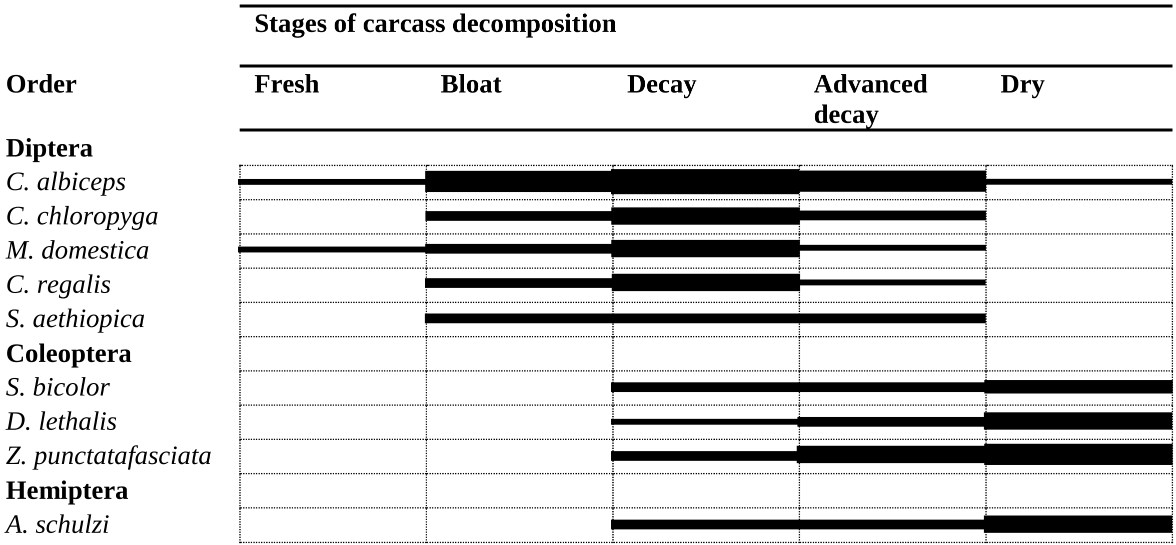



Each stage of decomposition is given the same amount of space in the table




The insect succession pattern in Marigat during the study period is shown in Table 4.5. Only dipterans and Coleopterans were observed in the carrion. Two species of dipterans (*C. alcibeps* and M. *domestica*) as well as Coleptera species (*Z. punctatafasciata*) appeared and conquered the carrion within the first 48 hours. All other dipterans and one species of Coleoptera (*S. bicolor*) appeared on the carrion between 24 to 48 hours at the bloat and stayed until the end of decay with highest numbers observed at decay stage. The other dipteran (*D. lethalis*) occurred first at decay stage and disappeared from the sample at advanced decay stage.

**Table 4.5: Insect species succession in each decomposition stage onpig carcass at Marigat site**




Each stage of decomposition is given the same amount of space in the table

Indicate <3 individuals present

Indicate 3-5 individuals present

Indicate 6-10 individuals present

Indicate 11-20 individuals present

Indicate >20 individuals present

### 4.3 Insect life cycle patterns during postmortem decomposition of pig carrion in Eldoret

Abundance of different development stages of insect sampled from each decomposition stage of the pig carrion at the site in Eldoret is presented in Table 4.6. Three species of Diptera (*S. senegalensis*, *C. albiceps* and *C. chloropyga*) did not start their life cycle in the fresh pig carrion but laid large batches of eggs after 24 hours in the bloat stage and continued laying more eggs at the decay stage. Low hatching rates of first larval instar (L1) as well as the second larval instar (L2) of these three dipteran species occurred at decay stage. At advanced decay stage, oviposition had declined while more eggs continued to hatch and first larval instars developed into second (L2) and third (L3) instars with low proportions of larvae pupating and some adult flies emerging. However at dry stage there was virtually no dipteran except few remaining prepupa and pupal stages that were latter established to have died. Meanwhile the other three species of dipterans (*S. cruentata*, *M. domestica* and *C. regalis*) commenced their life cycle on the carrion immediately after death during the fresh and bloating stages of carcasses by laying large numbers of eggs. The oviposition which continued until the decay stage. Hatching of these eggs into first larval instars occurred at slow rates at the bloat stage. At decay stage, the development of L1 to L2 larvae was high. Nevertheless, egg laying reduced at advanced decay stage as more of the L1 and L2 larvae developed into L3, with relatively smaller number of prepupae and pupae forming and even fewer numbers attaining adult stages.

Life cycle patterns of the various species of Coleoptera exhibited comon trend. They started their lifecycle at decay stage by laying large numbers of eggs. However development from eggs stahe to L1, L2 and L3 occurred at advanced decay stage culminating into sizeable numbers of prepupae and pupae. However, subsequent adults were observed to be low. At the dry stage of decomposition, there were very few visible lifecycle stages remaining for *Z. punctatafasciata* but there were relatively large number of prepupae, pupae and adults of *S. bicolor* and *D. lethalis* being observed in the samples.

The hemipterans generally started their life cycle stages at the bloat stage where they lay eggs in large batches, with low number of eggs hatching into L1 stage. Egg laying continued at the decay stage with numbers still large but more hatching into L1 and developing to L2 being noted. At advanced decomposition stage, there was a decline in oviposition as more of the earlier laid eggs and/ L1, and L3 develop into L3 in large numbers, with appreciable numbers reaching prepupa and pupa stage and very few attaining adult status. At the dry stage, large number of prepupa and pupa were observed but mergence to adult was quite low.

**Table 4.6: Abundance of each life cycle stage of insect species recorded during the five stages of decomposing pig carrion in Eldoret**

| Species | Fresh | Bloat | | Decay | | | Advanced decay (AD) | | | | | | | Dry (DR) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Eggs | Eggs | L1 | Eggs | L1 | L2 | Eggs | L1 | L2 | L3 | Prepupa | Pupa | Adult | Prepupa | Pupa | Adult |
| **Diptera** | | | | | | | | | | | | | | | | |
| *S. senegalensis* | 0 | 260 | 12 | 280 | 25 | 58 | 122 | 14 | 37 | 46 | 12 | 9 | 5 | 3 | 0 | 0 |
| *C. albiceps* | 0 | 340 | 20 | 370 | 45 | 49 | 210 | 45 | 48 | 72 | 14 | 12 | 32 | 2 | 1 | 1 |
| *C. chloropyga* | 0 | 320 | 26 | 360 | 41 | 46 | 235 | 43 | 32 | 67 | 21 | 15 | 17 | 1 | 0 | 0 |
| *S. cruentata* | 350 | 295 | 21 | 300 | 56 | 64 | 185 | 42 | 32 | 85 | 16 | 12 | 11 | 1 | 1 | 0 |
| *M. domestica* | 160 | 245 | 10 | 248 | 56 | 68 | 22 | 55 | 21 | 68 | 20 | 11 | 5 | 2 | 1 | 0 |
| *C. regalis* | 258 | 201 | 32 | 160 | 45 | 71 | 128 | 34 | 16 | 44 | 19 | 17 | 2 | 0 | 1 | 0 |
| **Coleoptera** | | | | | | | | | | | | | | | | |
| *Z. punctatafasciata* | 0 | 0 | 0 | 210 | 12 | 65 | 160 | 63 | 52 | 34 | 11 | 4 | 2 | 0 | 0 | 0 |
| *S. bicolor* | 0 | 0 | 0 | 185 | 15 | 65 | 180 | 55 | 30 | 18 | 8 | 3 | 5 | 10 | 8 | 5 |
| *D. lethalis* | 0 | 0 | 0 | 0 | 0 | 0 | 210 | 21 | 12 | 10 | 1 | 6 | 2 | 24 | 11 | 4 |
| **Hemiptera** | | | | | | | | | | | | | | | | |
| *A. schulzi* | 0 | 230 | 0 | 125 | 42 | 17 | 54 | 35 | 22 | 44 | 12 | 6 | 3 | 14 | 8 | 1 |
| *S. annulus* | 0 | 90 | 16 | 87 | 34 | 50 | 48 | 32 | 14 | 34 | 10 | 8 | 2 | 12 | 9 | 1 |
| *C. tetrastigma* | 0 | 125 | 25 | 130 | 44 | 68 | 35 | 20 | 12 | 35 | 8 | 5 | 1 | 7 | 5 | 1 |

Life cycle response of insect at different stages on pig carrion at Marigat is shown in Table 4.7. Two species of diptera (*C. albiceps* and *M. domestica*) started their life cycle on the fresh stage of the carrion and lay lots of eggs, which is carried on to the bloat and decay stage, however at the latter two stages, the eggs hatch into L1 and develop into L2. By the time these species reach Advance stage, the larval instars develop into large numbers of L3 and prepupae with less pupa and adults. The other three dipteran species (*C. chloropyga*, *C. regalis* and *S. aethiopica*) started their life cycle at the bloat stage of the pig carrion by laying lots of eggs after 24 hours and continued laying a lot of eggs at decay stage. Low hatching rates of first larval instar (L1) and second larval instar (L2) of these three species of diptera occurred at decay stage. At the advanced stage laying of eggs had reduced as more eggs continued to hatch and larval instars developed into more L2 and L3 with lower proportions reaching adults. However at dry stage there was virtually no diptera life cycle stage remaining.

Life cycle patterns of the species of Coleopteran were also similar except *Z. punctatafasciata*. As for *S. bicolor*, life cycle started at bloat stage by laying large number of eggs, which continued at decay and advanced decay stage, hatching of L1 started at bloat stage and development into L2 continued at decay stage, while advanced decay L3 was dominant followed by prepupa. In *D. lethalis*, life cycle stage started at decay stage through laying of eggs which produced low quantity of L2, while at advanced stage, the egg laying had reduced but development of L1 to L2 and L3 was more pronounced with sizeable number of pupa; the pupa persisted until dry stage with low adult population recorded. In *Z. punctatafasciata*, eggs were laid on fresh stage, decay and advanced stages but development into L1 started in bloat and continued to develop into L2 and L3 in decay and advanced decay.

The hemipterans were represented by *A. schulzi* which started their lifecycle at decays stage by laying eggs and the eggs started hatching to L1, is n advanced decay, laying of eggs reduced as they continued hatching to L1 and developing into L2 and L3 in large batches as well as prepupal stages but those that developed to adults were very few. This is the only group where the life cycle continued until the dry stage where there was large number of pupa and adult emergence when other taxa had left the carrion.

**Table 4.7: Abundance of each life cycle stage of insect species recorded during the five stages of decomposing pig carrion in Marigat**

| Species | Fresh | Bloat | | Decay | | | Advanced decay (AD) | | | | | | | Dry (DR) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Eggs | Eggs | L1 | Eggs | L1 | L2 | Eggs | L1 | L2 | L3 | Prepupa | Pupa | Adult | Prepupa | Pupa | Adult |
| **Diptera** | | | | | | | | | | | | | | | | |
| *C. albiceps* | 260 | 186 | 12 | 142 | 156 | 10 | 25 | 30 | 45 | 85 | 82 | 25 | 8 | 1 | 1 | 0 |
| *C. chloropyga* | 0 | 156 | 9 | 126 | 121 | 12 | 26 | 44 | 72 | 101 | 90 | 23 | 3 | 1 | 1 | 0 |
| *M. domestica* | 185 | 127 | 13 | 130 | 119 | 10 | 10 | 55 | 89 | 84 | 89 | 21 | 1 | 1 | 1 | 0 |
| *C. regalis* | 0 | 244 | 12 | 156 | 118 | 9 | 35 | 67 | 67 | 104 | 78 | 10 | 1 | 1 | 1 | 0 |
| *S. aethiopica* | 0 | 134 | 7 | 123 | 121 | 8 | 44 | 31 | 54 | 82 | 81 | 32 | 3 | 0 | 1 | 0 |
| **Coleoptera** | | | | | | | | | | | | | | | | |
| *S. bicolor* | 0 | 182 | 4 | 145 | 142 | 7 | 54 | 45 | 42 | 71 | 64 | 31 | 0 | 0 | 3 | 0 |
| *D. lethalis* | 0 | 0 | 0 | 121 | 12 | 0 | 42 | 60 | 78 | 92 | 90 | 22 | 2 | 0 | 2 | 3 |
| *Z. punctatafasciata* | 186 | 31 | 12 | 136 | 12 | 3 | 48 | 43 | 34 | 87 | 81 | 17 | 2 | 0 | 0 | 0 |
| **Hemiptera** | | | | | | | | | | | | | | | | |
| *A. schulzi* | 0 | 0 | 0 | 241 | 24 | 0 | 55 | 9 | 13 | 92 | 78 | 8 | 1 | 5 | 11 | 13 |

## 4.4 Role of environmental factors in insect succession during postmortem decomposition of pig carrion in Eldoret

Data on the environmental variables that were measured and averaged over the period of each stage of carcass decomposition are shown in Figure 4.4. The data included ambient temperature (°C), soil temperature (°C), relative humidity (mmHg), rainfall (mm), and cloud cover (%) and wind speed (km/h). Ambient and soil temperatures were significantly ($P < 0.05$) higher in Marigat than in Eldoret and displayed significant differences ($P > 0.05$) during the decomposition stages. Relative humidity, rainfall, and humidity were consistently higher in Eldoret than in Marigat and showed significant changes when the carcass was in the decomposition stage. Wind speed was higher in Marigat throughout the decomposition stages being similar in fresh, bloat and decay stage but increased in advanced and dry stages in Marigat but reduced over the same stages in Eldoret. This significantly affected both landing and resident time by the indicator insects on the pig carcass.

The relationships between insect abundance at the stages of decomposition relative to the environmental variables are shown in Figure 4.5. Three principle factors (eigen values > 1) were extracted to explain the variability in the principal component analysis (PCA) and together, the factors explained 72.3% of the total data variance. The abundance of insect at bloat, decay, and dry stages and advanced decay were positively associated with ambient temperature, soil temperature, relative humidity and wind speed. There was strong coupling observed between ambient temperature and soil temperature with insect abundance at decay stage. Precipitation was the only variable affecting insect abundance negatively at fresh stage.

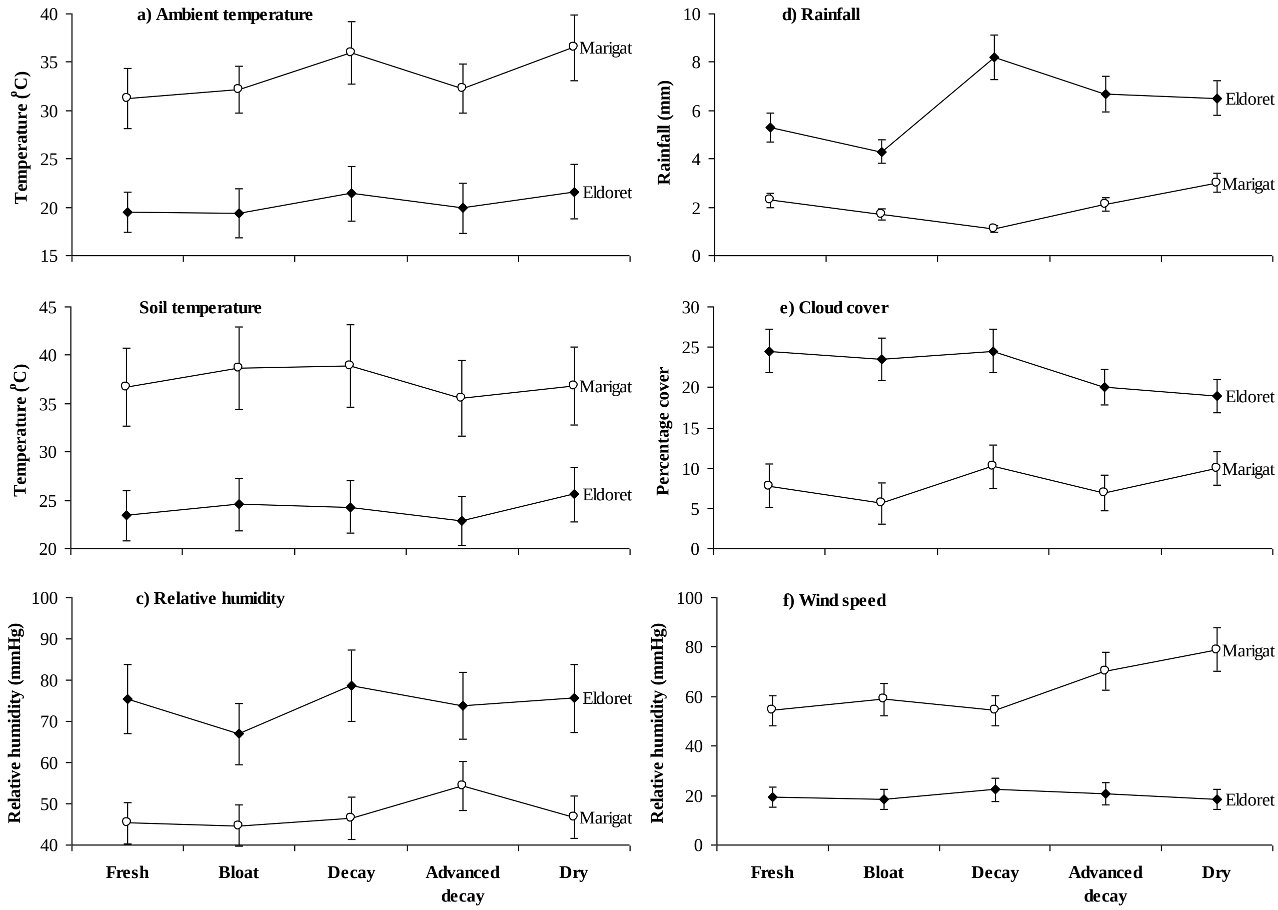



**Figure 4.4: Data on the environmental variables measured in Eldoret and Marigat during the experimental period.**

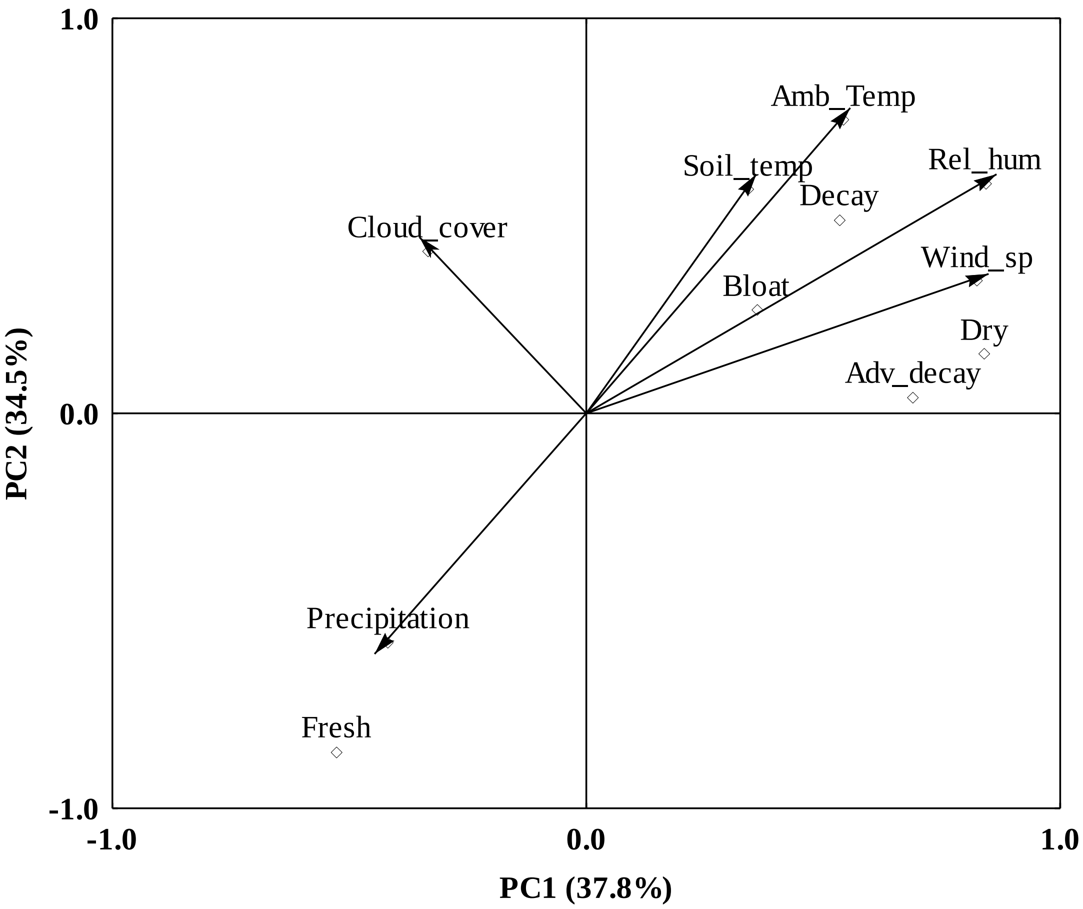



**Figure 4.5: Principal Component Analysis (PCA) of abundance of insects at different stages in carrion relative to environmental variables at Eldoret and Marigat sites, Kenya**

Meanwhile the individual relationships between the abundance of individual insect species at the each stages of decomposition relative to the environmental variables are shown in Figure 4.6. At fresh stage, abundance of *C. chloropyga* was positively influenced by soil temperature, *M. domestic* and *C. cruentata* by ambient temperature while relative humidity influenced *C. regalia* and *G. maculate*, with wind speed influencing the abundance of *C. albiceps*. At bloat stage, the abundance of *M. domestica* and *S. cruentata* were positively related to ambient temperature while soil temperature and precipitation positively affected *C. chloropyga*, with *G. simplex* and *G. maculata*. At the decay stage, soil temperature, precipitation and relative humidity affected the abundance of *M. dometica*, *S. cruentata*, *C. chloropyga*, *G. simplex*, *G. maculata* and *C. regalis*. On the other hand, ambient temperature, cloud cover and wind speed influenced the abundance of *Z. punctatafasciata*. At the advanced decay stage *S. cruentata*, *C. chloropyga*, *G. simplex*, *G. maculata* and *C. regalis* were positively influenced by relative humidity and precipitation; soil temperature, cloud cove and ambient temperature positively influenced the abundance of *Z. punctatasciata* and *M. domestica* while wind speed influenced *C. albiceps*.

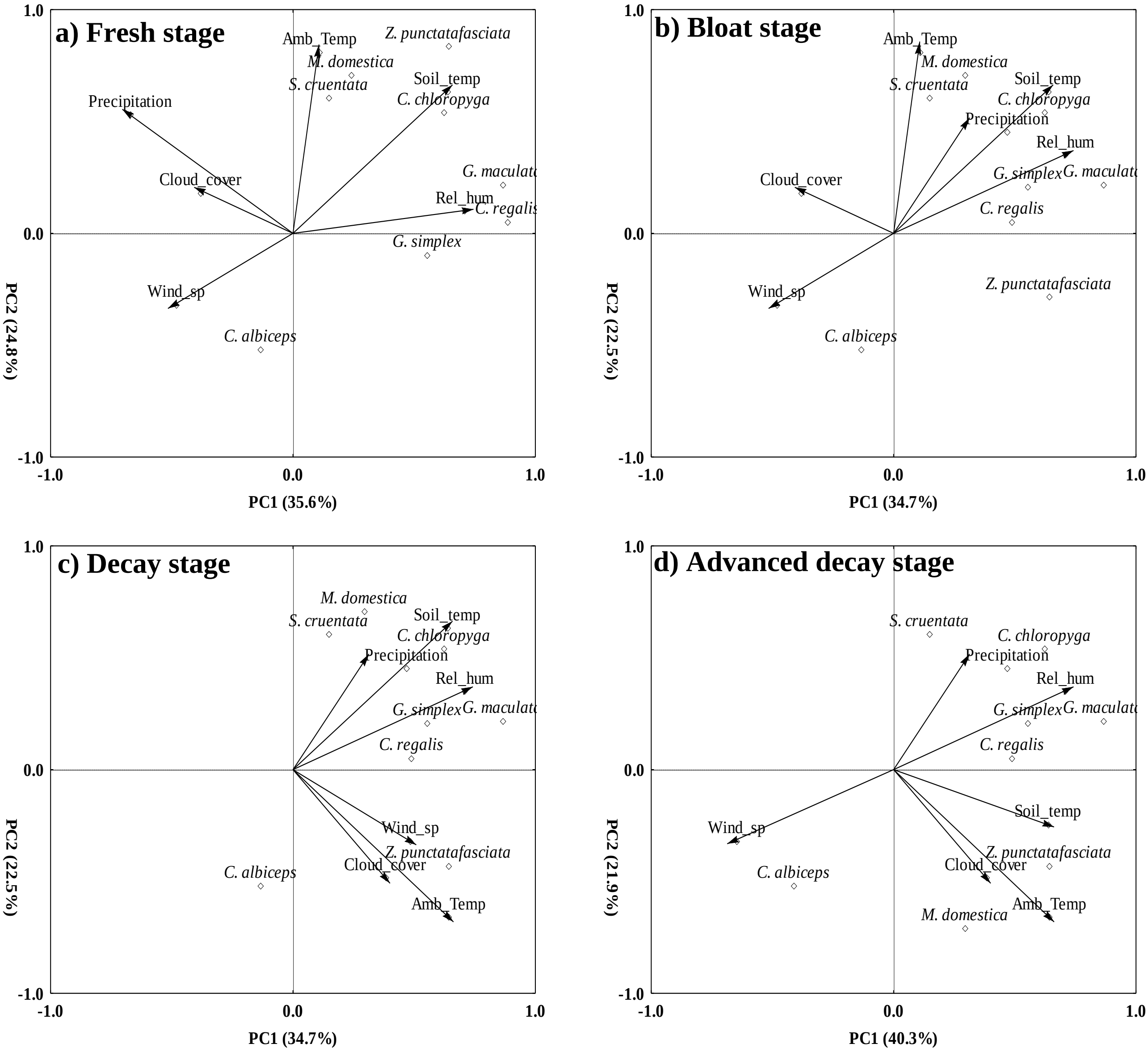



**Figure 4.6: Principal Component Analysis (PCA) of abundance of individual insect species in carrion at each stage of decomposition relative to environmental variables at Eldoret and Marigat sites, Kenya**

# CHAPTER FIVE

# DISCUSSION

## 5.1 Gross carcass decomposition and environmental conditions

Although the pattern of decomposition is recognized globally, this is the first study to be done in Kenya, using pig carrion as a model for decomposing humans. The five decomposition stages (fresh stage, bloat stage, decay stage, advanced decay and dry stage) were observed in pig carcasses. These observations are similar to those in other studies on pig carrion decomposition (Barton & Bump, 2019; Feddern *et al.*, 2019; Matuszewski *et al.*, 2019).

This study also determined that the decomposition occurred over a period of 26 to 36 days and was rapid initially and only bones and skin remained by day 16 and slowed down at latter stages. The number of days recorded in current workwere relatively fewer compared to those reported for similar experiments elsewhere during the summer, for example 38 days in Hawaii (Rysavy & Goff, 2015), 40 days record in Western Australia (Deo *et al.*, 2019) and Brazilian savanna (De Faria *et al.*, 2018), These value are lower than the 83 days reported in Colombia (Valverde-Castro *et al.*, 2017). These values are also higher than 14 days reported to complete decomposition of pig carrion in Malaysia (Silahuddin *et al.*, 2015). It has been stated that differences in carrion decomposition may be subject to environmental conditions at the time or differences in geographical range which may be offset by a number of environmental variable working in concomitance (Pushkin, 2015). High rate of decomposition of pig carrion observed in the current study agree with several studies in the tropics due to presence of favorable environmental conditions. These optimal conditions favour rapid bacterial, fungal and protozoan colonization and breakdown of the body tissues (Sanford, 2017).

After fresh, bloat and decay stages, which were similar at the two sites, the advanced decay and dry stage was established to be faster in Marigat than in Eldoret. This was attributed to higher ambient temperature and lower humidity as reported for other studies elsewhere (Tomberlin *et al.*, 2017; Singh *et al.*, 2018; Somavilla *et al.*, 2019). Current data also show that there are spatial and temporal variation in post-mortem decomposition that are probably due to of similar environmental variables, mainly temperature. Indeed this study established that ambient temperatures at Marigat were consistently higher than at Eldoret. The internal temperatures of the pig carrion at Marigat and at Eldoret at all stages of carrion decomposition followed a similar pattern.

There was a gradual increase in the internal temperature of the carcasses, from the early stages of decay to towards decay to a maximum value at advanced decay stage before finally dropping to a lower level during the progression of dry stage. The increased temperature during the carrion decomposition is suspected to be due to mass aggregation of the insect larvae and the subsequent frenetic activities of micro-environmental conditions. It is often probable for temperature to surpass the upper threshold for the arthropods resistance, as observed in this stage. As recently observed, a maximum temperature of 49°C has been established in carcasss which was consistently higher than the ambient temperature ranging from 9 to 22°C (Von Hoermann *et al.*, 2018; Zeariya & Kabadaia, 2019). Also similar to the current study, an internal temperature of 44–49°C was recorded in a sheep carcass in an area whose ambient temperaturerange from 16 to 18°C (Maramat & Rahim, 2015). Based on carcass of domestic pig *Sus scrofa*, there have been a record of a remarkable internal temperature ranging between 45–50°C, nine days subsequent to exposure (Mądra *et al.*, 2015). In another study using pig carcasses a maximum temperature ranging between 44- 52°C thermally increasing 20°C above threshold (Jarmusz & Bajerlein, 2019). The drop of internal temperature towards the end of the advanced decay stages in both regions could therefore be due to post-feeding larval activities during their migration to the pupae phase. It could also be due to a drop in ambient temperatures, though this was not the case at the two experimental sites in this work.

### 5.2 Insect succession patterns during postmortem decomposition of pig carrion in Eldoret

During the study, of insect fauna on pig carcasses and the pattern of colonization at Eldoret and Marigat sites revealed an elaborate and unique arthropod species colonizing pig carcass at the study area as: (I) Fresh – Diptera (*C. cloropyga*, *S. cruentata* and *M. domestica*) and Hemiptera (*A. annulus* and *C. tetrastigma*) (II) Bloat – all Diptera species and Hemiptera. (III) Decay – all orders studied (IV) Advanced Decay – all orders studied and (V) Mainly Coleoptera (beetles). The current study is similar to previous studies where carrion succession in tropical O’ahu, Hawaii on carcasses of domestic cats was characterized by presence of groups of insects that were found in similar studies conducted in other regions, however, there was avariation in the species compositiont (Rysavy & Goff, 2015).

A notable observation was that members of other families including silphid and nitidulid beetles, which are common on carrion in other studies (Sebastião & Prado e

Castro, 2018) were absent. In that study, the earliest colonizers were *Lucilia cuprina* and *Sarcophagula occidua*. In this study the overall abundance of taxa at Eldoret and Marigat were lower than most of abundance reported in temperate, continental regions (Sharanowski *et al.*, 2008; Sutherland & O'Donnell, 2018) but the pattern where pioneer colonization by blow flies on the decomposing carrion was respected. As a basis for comparison, in Tennessee, a total of 217 insect species were found in dog carrion, here calliphorids and *Lucilia coeruleiviridis* pionnered the colonization (Cobaugh *et al.*, 2015). Meanwhile in another town in Canada (Saskatchewan), calliphorids of the species *Cynomya cadaverina*, *Phormia regina* and *Protophormia terraenovae* colonized the pig carcasses during the summer (Matuszewski *et al.*, 2016). Thus, reported variation in insect colonization patterns and species composition on carcasses under different environments underlines the necessity of duplicate studies at different spatial scale.

The dominance of Calliphoridae family as pionner colonizers is may be due to high perspicacity of decay odors by the insect from long distance of the carcass (Perrault *et al.*, 2015; Liu *et al.*, 2016). In the study, the blow flies (Diptera: Calliphoridae) at stage III and IV were the second to settle on the carcass which concured with previous studies of Beuter and Mendes (2013) conducted in Paramo, Colombia. In the current study, *C. chloropyga* and *S. cruentata* were the first species to settle on the carcass as early as 30 minutes following death. The higher temperatures recorded in Marigat permitted carcasses visits by two adult flies, *C. albiceps (*Diptera: Calliphoridae) that oviposited. The presence of this species immediately after setting up the carcass in the field in Marigat is associated with its ability to be tolerant to high temperatures encountered in Marigat during the study.

Based on the result of the foregoing study, dipteran flies arrived first on the carrion followed by flesh flies and then coleopterans (beetles) came last on the carrion. Ants were also obtained from carrion throughout the decomposition period, feeding on both carrion and other insects (Formicidae), however, they did not seem to be relevant in the current study since these are always described as “accidental visitors”. At the advanced stage of colonization, the beetles domited. Therefore based on the current study, it was observed that forensically important arthropods belong to order Diptera (flies) and Coleoptera (beetles) and few other species of Dermaptera. The blowfly larvae are necrophagous, meaning they feed on decomposing tissue (Goff, 2009). The role of necrophagous dipteran likes fleshflies and beetle can also not be overlooked as they appear to eliminate soft tissue from decomposing carrion and therefore becomes one of the most important factor that must be considered in the carrion decomposition ecology.

It was observed that *C. albiceps* dominated the blowfly community and were conspicuous at all stages of decomposition which concurs with other studies in the tropical region (Frątczak & Matuszewski, 2016; Guerroudj *et al.*, 2017). It is possible that the avid feeding nature of this species mostly likely contributed to the contributes to the high abundance of this species (Gunn, 2019). It was also notable that advanced decay stage reported most of the insects, which provided more resource to the insects to encourage colonization. Pephaps the odour from the decaying carcase together with the volatile organic acids and compounds were soufficien to nourish the insects.

### 5.3 Insect development pattern during postmortem decomposition of pig carrion in Eldoret

Arthropod lifecycle strategies are important in forensic studies since relating the development pattern of arthropods and the time it takes to complete this process on the carrion will avail useful information to estimate the time that death occurred (Armstrong *et al.*, 2016; Al-Qahtni *et al.*, 2019). In Kenya however, the lifecycle assessment of the arthropods on carrions has rarely been done. This is therefore the first study to follow a life cycle development of various species of arthropods on pig carrion.

Adult flies oviposit on decomposing remains within a few hours after death (Brundage *et al.*, 2014; Bugelli *et al.*, 2018). During the study, three species of dipterans, *S. cruentata*, *M. domestica* and *C. regalis* commenced their life cycle by laying eggs on the carrion in the field. Presence of eggs of these species indicates that the carrion is still fresh or at early blot stage, during which oviposition continued. Previous studies have indicated that some dipteran species exhibit very fast development in their life cycle and will lay eggs on arrival to ensure that they produce several generations on the carrion (Galal *et al*., 2009; Grassberger and Frank, 2014; Mądra *et al*., 2015). Hatching of these eggs into first larval instars occurred at slow rates at the bloat stage and after about 30 to 40 hours on the carcass, most of these eggs had hatched into L1 and developed to L2 instars. Insect species whose development patterns involve aggressive feeding along the decomposition pathway will develop faster and may eventually act as predators on the development stages of other insects that oviposit later, therefore making it difficult to accurately identify the development stages of other insects on the corpse (Amendt *et al*., 2004). In this study, three species of Diptera, *S. senegaeinsis*, *C. albiceps* and *C. chloropyga* were found to be the second group of colonizers that started their life cycle by laying eggs on the carcass at the bloat stage, approximately 24 after death. After about 48 hours, the eggs hatched into first larval instars (L1) and further, after 60 hours they moulted into second larval instars (L2), at the decay stage. Thse larvae pupated and the adults emerged at the advanced decay stage. Nevertheless, egg laying declined at the advanced decay stage as more of the L1 and L2 instart larvae developed to L3 with relatively smaller numbers of prepupae and pupae and even fewer appearing to reach adult stages. This is probably due to diminishing amounts of soft tissues that is fed on in the pig carrion (Dekeirsschiete *et al*., 2009; Catts and Haskell, 2015).

The last species to start their life cycle that were observed in this study were carrion beetles (Coleoptera)which started at decay stage by laying large numbers of eggs. However, most of the development from eggs to L1, L2 and L3 occurred during the advanced decay stage culminating to sizeable numbers of prepupae and pupae at advanced and dry stage. They colonize the carcass at latter stages to start their life cycle because they breakdown and recycle organic matter as a source of food (Kalinova *et al*., 2009). At the dry stage there were very few visible lifecycle stages remaining for Z. *punctatafasciata* but there was relatively large number of prepupa, pupa and adult *S. bicolor* and *D. lethalis* were present in relatively large numbers.

When using life cycle stages of insects to estimate important forensic parameters, caution is emphasized. For instance, *Thanatophilus micans* (Coleoptera:Silphidae) was present on carrion within 24 hr of death but its life cycle is longer than those of sympatric flies (Villet and Amendt, 2011). This bettle species feeds on the eggs and maggots of other arthropods, implying that if there are more than one species of flies in the maggot stages, presence of these development stages may be obscured by interspecies predation. Also the adults of *Trox* sp. feed on the pupae of many insect species and therefore may interfere with the development patterns of these insects (Masan, 2013). During the larval stage, *C. albiceps* are facultative predators and therefore can alter the composition of species present in the carcass and cause a stronger impact than the other blowfly species (Faria and Godoy, 2001). Besides being a predator, larvae of *C. albiceps* cannibalize each other (Faria *et al*., 2004). This represents the classical 'exterminator' mechanism of ecological succession in insects and may therefore make interpretation of the life cycle presense of insects in corpses/carcasses rather complex.

### 5.4 Role of environmental factors in insect succession patterns during postmortem decomposition of pig carrion in Eldoret

The weather conditions were recorded during the study period. Eldoret was generally hot and humid while Marigat region was hot and dry. These conditions contributed to the accelerated decomposition process, which is in agreement with the findings of other studies (Rutty, 2001; Sharanowski *et al.,* 2008; Richards and Villet, 2009; Sarthak *et al.*, 2016). In this study, the ambient and soil temperatures were higher in Marigat than in Eldoret.

The relative humidity and rainfall were consistently higher in Eldoret than in Marigat while wind speed was higher in Marigat throughout the study period. Previously, studies have established that each of these individual factors affect the rate of decomposition and arthropod succession on pig carrion (Archer, 2004; Battán

Horenstein *et al*., 2010; Frederickx *et al*., 2012; Vasconcelos *et al*., 2013; Matuszewski and Mądra, 2015; Oliveira *et al*., 2016; Barton *et al*., 2017; Mashaly, 2017; Shaalan *et al*., 2017).

Nevertheless, the role of individual meteorological factors was not tested on the decomposition and succession stage because the variations were only for two regions. Furthermore, temperature and relative humidity conditions at the two sites exhibited little variation throughout the study period. Thus it was not possible to demonstrate the influence of these factors on the presence of insects on the carcass or on the decomposition process itself. Moreover, the relationship between an individual environmental factor and the number of individuals collected is mostly evident when there is significant seasonal variation (Carvalho and Linhares, 2001). Therefore, the association between these environmental factor and decomposition as well as insect succession was done based on the multivariate techniques.

During the study, the abundance of insect at bloat, decay, and dry stage and advanced decay were positively associated with ambient temperature, soil temperature, relative humidity and wind speed; ambient temperature and soil temperature affected insect abundance at decay stage while precipitation affected insect abundance at fresh stage. Temperature affects the reaction rates and development of bacteria (Simmons *et al*., 2010). Level humidity influences the extent of water loss of exposed tissue, in particular at the skin surface (Mann *et al*., 2011).

Thus, in arid climates or in circumstances of extremely low humidity dehydration of the skin occurs rapidly producing a hard and leathery covering, impermeable to the transfer of water from underlying tissues to the surface (Aturaliya and Lukasewycz, 1999) Further, precipitation or rainfall introduces moisture or water into the environment, which is essential for the biochemical reactions that causes tissue degradation (Tabor *et al*., 1997). Previous studies have established that variation in rainfall patterns affect carrion decomposition rates and subsequently the number of days to complete decomposition (De Carvalho and Linhares, 2001; Kočárek, 2003; Shi *et al*., 2009). In another study, Kyerematen *et al*. (2013) established that pig carrion completely decomposed in 16 and 24 days for the dry and wet seasons, respectively.

In the current study, it was established that at each decomposition stage, each environmental variable affected the abudance and succession of different insect species. For instance at fresh stage, abundance of *C. chloropyga* was positively influenced by soil temperature, *M. domestica* and *C. cruentata* by ambient temperature while relative humidity influenced *C. regalis* and *G. maculata*, with wind speed influencing the abundance of *C. albiceps*. At bloat stage, the abundance of *M. domestica* and *S. cruentata* were positively related to ambient temperature while soil temperature and precipitation positively affected *C. chloropyga,* with *G. simplex* and *G. maculata*. At the decay stage, soil temperature, precipitation and relative humidity affected the abundance of *M. dometica*, *S. cruentata*, *C. chloropyga*, *G. simplex, G. maculata* and *C. regalis*. On the other hand, ambient temperature, cloud cover and wind speed influenced the abundance of *Z. punctatafasciata*. At the advanced decay stage *S. cruentata*, *C. chloropyga*, *G. simplex*, *G. maculata* and *C. regalis* were positively influenced by relative humidity and precipitation; soil temperature, cloud cove and ambient temperature positively influenced the abundance of *Z.*

*punctatasciata* and *M. domestica* while wind speed influenced *C. albiceps*. These results indicate that each species of insects have favourable conditions that allow them to be active and the rate of activity can be enhanced by a combination of environmental factors working together, rather than a single environmental variable.

# CHAPTER SIX

# CONCLUSIONS AND RECOMMENDATIONS

## 6.1 Conclusion

The study established five stages during the decomposition of pig carrion comprising of fresh, bloat, decay, advanced decay and dry based on the physiological changes of the carrion. It was also established that decomposition occurred over a period of 26 to 36 days with latter stages of advanced decay and dry taking place at faster rate in Marigat than in Eldoret due to the prevailing environmental conditions. Internal temperature of the carrion was also higher in Marigat than Eldoret at developmental stages.

Insect succession pattern in Eldoret and Marigat were similar but each differed in their abundance at each stage. The following species were prevalent in the various stage: (I) Fresh – Diptera (*C. chloropyga*, *S. cruentata* and *M. domestica*) and Hemiptera (*A. annulus* and *C. tetrastigma*). (II) Bloat – all Diptera species and Hemiptera (III) Decay – all orders. (IV) Advanced Decay – all orders and (V). Mainly Coleoptera (beetles). The succession pattern although similar, varied slightly under two environmental conditions.

The life cycle response on the carrion followed similar pattern on Eldoret and marigat albeit at different time intervals. Several species of dipterans (*S. cruentata*, *M. domestica* and *C. regalis*) were the first to lay eggs and commence their life cycle immediately after death on the fresh and on bloating stage followed by three species of diptera (*S. senegalensis*, *C. albiceps* and *C. chloropyga*) which started their life cycle at the bloat stage. After about 48 hours, they hatch into first larval instar (L1) and further after 60 hours they moulted into second larval instar (L2) which is at the decay stage. Their life cycle appear completed at the advanced stage. The last species to start their life cycle were Colepterans (carrion beetles) which started at decay stage by laying eggs and developing through their L1, L2 and L3 stages at advanced decay stage before completing their life stages at dry stage.

During the ambient and soil temperatures was higher in Marigat than Eldoret; relative humidity, rainfall, humidity were consistently higher in Eldoret than Marigat while wind speed was higher in Marigat throughout the decomposition stages. The abundance of insect at bloat, decay, and dry stage and advanced decay were positively associated with ambient temperature, soil temperature, relative humidity and wind speed; ambient temperature and soil temperature affected insect abundance at decay stage while precipitation affected insect abundance at fresh stage. Moreover, when analysis was done at each decomposition stages, it was established that at each stage the abundance and success of each environmental variable affected different species.

From the results of this study, it is promising that with further similar data collection to minimize deviations in species abudance and succession, we shall be able to establish robust and reliable use of forensic entomology in Kenya.

### 6.2. Recommendations

1. The application of the knowledge of forensic entomology in Kenya for routine criminal investigations faces dauting tasks. Therefore more studies should be conducted in Kenya at different ecological zones to provide the baseline data that appears to be missing and can form the basis for future refeerence.

2. Future studies of the carrion decomposition under different death scenarios are important since the current study made largely generalistic assumption that dead coprse wll be found in the field. However there are a number of deaths scenarios that may be investigated to provide variations in the nature of death worth investigating.
3. To properly understand insect succession, databases of insects associated with carrion should be compiled for each regions in Kenya, under various factors that may affect succession so that it becomes easy to fully synchronize succession pathways into a theoretical understanding.
4. Future studies may address several areas in which progress is possible to make forensic entomology more reliable. Improvement of forensice science models and theoretical frameworks.

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**APPENDIX**

# APPENDIX I: SIMILARITY REPORT



## Turnitin Originality Report

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