EFFECTS OF DIFFERENT KILLING METHODS ON *CRICETOMYS GAMBIANUS* IN ASSESSING INSECT FAUNA SUCCESSION AND DETERMINATION OF POSTMORTEM INTERVAL

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TITLE PAGE

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CERTIFICATION

Eze, Sabina Chioma a postgraduate student in the Department of Zoology and Environmental Biology with registration number PG/M.Sc./15/78822 has satisfactorily completed the course and research work requirements for the award of Masters Degree (M.Sc.) in Entomology and Forensic Science.

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DEDICATION

I dedicate this work to my Elder brother, Dr. Eze Melletus Ugonna.

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ABSTRACT

A study was carried out to determine the effects of different killing methods of rat on decomposition, insect succession and determination of postmortem interval. Nine giant rats (*Cricetomys gambianus*) were used to simulate violent death, natural death and suicide death. One rat was slaughtered to simulate violent death; another was killed by oxygen exclusion to simulate natural death. The third rat was killed by oral injection of 5 ml of 2, 2-dichlorovinyl dimethyl phosphate (DDVP) (common home pesticides) to simulate suicide death. Each of the killing methods was replicated three times and deposited in three sites within University of Nigeria, Nsukka. Decomposition took place under mean temperature and mean relative humidity of 31.05 °C and 71.02 % respectively. The mean decomposition period for violent, natural and suicide death were 30.33±4.67, 48.33±6.67 and 60.00±5.77 days respectively. The arthropod samples collected in this study was 5036 individuals, consisting of 10 orders, 23 families and 50 species. Among the necrophagous families collected were: Calliphoridae, Sarcophagidae, Muscidae, Formicidae, Phoridae, Oestridae, Dermestidae, Cleridae, Curculinidae, Chrysomelidae, Scarabaeidae, Trogoidae, Histeridae, Carabidae, Cucujiformia, Vespidae, Rhipiceridae, Gryllidae, Erebidae, Blattidae, Belostomatidae, Araneae and Rhinocricidae. The arthropods visited the decomposing carcass in the following successional pattern: Formicidae visited after 5 mins of death; Calliphoridae visited after 29 mins; Muscidae visited after 20 mins; Sarcophagidae visited after 2 hrs 45mins; Histeridae visited after 2 days; Dermestidae and other families visited after 4 days and beyond. The greatest number of species was observed in natural death (36 species), followed by suicide death (35 species) and violent death (27 species). The violent and natural death had similar PMI of 9 days after death but in suicide death PMI was 25 days after death. The adult Diptera used in calculating PMI were *Chrysomyia albiceps*, *C. chloropyga* and *Lucillia sericata* in violent death, natural death and suicide death respectively under the fluctuating laboratory temperature and relative humidity range of 23°C - 32°C and 69% - 82% respectively. The PMI of 25 days was caused by overdose of the chemical when compared to the propotion of the size of the animal (rat). The investigation revealed that death due to suicide (chemical intoxication) can delay decomposition and results in PMI error.

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CHAPTER ONE
INTRODUCTION AND LITERATURE REVIEW

1.0 Introduction

Forensic entomology can be defined as the use of insects and their arthropod relatives that inhabit decomposing remains to aid legal investigations (Rafael and Liliana, 2013; Suhad *et al.*, 2016). According to Amendt *et al.* (2011), forensic entomology is the science of collecting and analyzing insect evidence to aid in forensic investigations. It is the study based on the principle of ecological succession of insect communities as biological indicators associated with the dead body for calculation of post mortem interval (PMI) (Muhammad *et al.,* 2013). It is also the application of science to serve the purposes of the law (Nick and Dando, 2015). According to Harvey *et al.* (2016), forensic entomology is the use of insects and other arthropods to estimate the minimum time elapsed since death, referred to as minimum postmortem interval ($_{min}$ PMI). Forensic entomology is a growing field of study, incorporating an entomologist's expertise in insects including their identification, life cycles and habitats, with the aim of law enforcement. Insect life cycles act as precise clock, which begin within minutes of death; hence it can be used in forensic studies.

According to Lord and Stevenson (1986), Forensic entomology is divided into three components:

Urban forensic entomology: a legal proceeding involving insects and related animals that affect man-made structures and other aspects of the human environment.

Stored products entomology: proceedings involving insects infesting stored commodities such as cereals and other kitchen products.

Medico-legal entomology: sometimes termed "forensic medical entomology", and in reality "medico-criminal entomology" (because of its focus on violent crime). It relates primarily to determining the time of death (postmortem interval), site or place of death, mode of death, cases involving sudden death, traffic accidents with no immediately obvious cause, possible criminal misuse of insects, abuse on children and elderly (Lord and Stevenson, 1986).

Determining when a victim died is often one of the most important questions to be answered in a homicide, as it helps to focus the police investigation into the correct time frame, greatly increasing police efficiency. Time of death becomes particularly important when the victim has been dead for some time. Most pathologists will not estimate time since death beyond 72 hours and many will not go beyond 24 hours. However, homicide victims, by the very nature of the crime, are frequently not discovered for days, weeks, months or more after death. In such cases, forensic entomology is the most reliable and frequently the only method of determining time since death. More recently, forensic entomology is also being used in the early postmortem interval. Insects are attracted to a body within minutes. The developmental rates of blow flies (Diptera: Calliphoridae) being the first insects that is attracted to death can be used to determine postmortem interval. There are two ways of determining post-mortem interval; use of insect growth and development and use of successional waves of insects (Simon Fraser University Museum of Archeology and Ethnology, 2010). When an entomologist correctly determine the postmortem interval in the cases of death crime, he/she can write a valid report which can be presented in the court of law for justice to rein or it can form a baseline information for other police investigations. Furthermore, forensic science (FS) has contributed greatly to the evidence-base of criminal investigations. It has triumphed in exposing miscarriages of justice. It has evolved from a supporting role to become a front-line analytic tool, often directing the efforts of investigators (Nick and Dando, 2015). It plays essential role in the effectiveness of the Criminal Justice System (CJS). To sustain confidence in forensic information, it is important to consider how it is produced: not only in terms of its scientific accuracy, but also to show that sample are handled appropriately to avoid contamination, and to demonstrate the impartiality and integrity of the staff undertaking the work. It is notable that academic, investigative, political and media attention on forensic science has focused almost exclusively on crime scene investigative input and prosecution process outcomes in the courtroom. Moreover, the human examiner plays a critical role in many forensic domains (often it is the human examiner who is the ‘instrument of analysis’). Forensic work often involves human perception, interpretation, evaluation, judgments and decision making. Therefore, forensic work is shaped by, and depends on, cognitive and human factors hence, the need for expertise and more experience on this profession. These underpin most aspects of forensic work from the initial collection and evaluation of data throughout the work in the forensic laboratory where evidence is interpreted and conclusions are

reached to the presentation in court and to other end users who are the customers of forensic work maximizing the use and benefit of forensic science while minimizing cognitive bias within forensic work it requires educating practitioners and implementing cognitive best practices (Itiel, 2015). The implications for court and the criminal justice system are that the forensic evidence may be overstated, and its uncertainties and limitations concealed. This is why there is need for consideration of many factors affecting PMI such as temperature, humidity, mummification, geographical location, burial, toxic chemicals, open wound, body size, clothing and exertion prior to death. When records have been established on each of the above factors of death that affect decomposition and PMI determination, valid and precise evidence can be presented in the court.

1.1.1 **Justification of the study**

Currently the rate of crime in Nigeria is alarming and the police lack the scientific means/evidence of investigating cause, place and time of death. Many innocent people therefore fall victim to such allegations. Many atimes, assassins kill people and take them to another location to avoid being suspected by police and the police will end up arresting the people around the vicinity where the dead body has been transferred. The increasing rate of suicide in our society is also alarming and there is need for investigating the proper cause of death of such subject as a cadaver can neither talk nor give proper information that could enhance investigations. There have been several cases where innocent girls are intoxicated with cocaine, drugs, alcohols or other chemicals in birthday parties, Valentine celebrations and other occasions; after which they are raped by many boys which subsequently result in death of such victims. There have been also cases where frustrated individuals commit suicide by drinking any of the common household chemicals. These kinds of crimes that are fast growing in our society have necessitated this research to assist the law enforcement agents in investigating crimes using insect evidence. Moreover, human cadaver are not always available for this kind of study therefore mammals are used to study different methods of death /types of death since they have similar decomposition rate as human cadaver (Goff, 2009b). Little of this work has been done in Nigeria and there is need to get valid data for estimation of post mortem interval because data obtained from one region may not be used in another as PMI are affected by geographical locations due to change in abiotic factors like temperature, humidity, and rainfall which affects

insects colonization and development (Kapil and Reject, 2016). There has been no reported work of this kind using African giant rat (*Cricetomys gambianus),* in this environment.

1.1.2 General objective of the study

The general objective of this study is to investigate the effects of different killing methods on *Cricetomys* gambianus for determination of postmortem interval and for the assessment of insect faunal succession.

1.1.3 Specific research objectives

The objectives of the study were to:

- investigate the effects of different killing methods on insect fauna during the decomposition of the giant rat (*Cricetomys gambianus*).
- determine the species composition and relative abundance of insects which colonize the decomposing African giant rat.
- determine time since death or post mortem interval using maggot age.

1.2. Literature Review

1.2.1 Insects of forensic importance

There are many arthropods that colonize carcass on the crime scene but some are not very important in the forensic studies. The most useful insect orders of forensic importance are the order Diptera (mostly Calliphoridae, Scarcophagidae, and Muscidae) and the Coleoptera (Amendt *et al.,* 2004). Arthropods that colonize the carcass in a crime scene are necrophagous species, predators and parasites of necrophagous species, adventives species and the accidental species. Ewuim and Abajue (2016) also documented the common arthropod families and species of forensic importance both in Nigeria and elsewhere and the development of forensic entomology so far in Nigeria.

- **Necrophagous species**

Necrophagy is the feeding behaviour of an organism that eats carrion from another animal that it did not kill. Necrophages feed and breed on the carrion itself. These species typically occur in succession and respond to decompositional changes of the carcass. These species are often the most important in providing useful forensic information (Lord, 1990; Amendt *et al.,* 2004). The Calliphoridae are the most prominent necrophages. Sarcophagids, muscids and piophilids may

also be necrophagous. Silphids, clerids and dermestids also feed and breed within the carrion (Ginger, 2005; Amateur Entomologist's Society, 2016). Diptera and Coleoptera comprise about 60% of the total necrophagous fauna found on carrion (Greenberg, 1991). Of the estimated 23 fly and 19 beetle families commonly associated with carrion, there are only about 10 families considered to be worth studying in depth in terms of their forensic value. Among the Diptera, the main families are the Calliphoridae, Sarcophagidae, Muscidae and Piophilidae. Calliphorid larvae, and to a lesser degree, sarcophagids and muscids, are the primary flies responsible for the majority of carrion decomposition during the earlier stages of decomposition. In the Coleoptera, the main families are the Silphidae, Staphylinidae, Cleridae and Dermestidae and to a lesser degree, geotrupids and trogids (Ginger, 2005). Many of the beetle species associated with carrion are predators of maggots and only a few are true carrion feeders. Some important families of necrophagous species are:

Calliphoridae

Blow flies (Fig. 1) are two-winged flies belonging to the Order Diptera and family Calliphoridae. They feed on decomposing carrion but when carrion is not available, these insects may feed on dung and other kinds of decomposing refuse (Gillott, 1995; Ginger, 2005, Jens *et al.,* 2010). They are very important in forensic entomology because they are the first insects that visit the carcass or crime scene hence is used to determine the post mortem interval. Some common names for blow flies are bluebottle, greenbottle, black blow flies or carrion flies (Terry, 2016). Blow flies subfamilies include Chrysomyinae, Calliphorinae and Luciinae. Calliphoridae are distinguished from other metallic or partly metallic calyptrate Diptera by the presence of row of bristles on the meron, absence of a prominent subscutellum and plumose (hairy) arista antenna. They are medium to large flies (4-16 mm in length) with metallic blue or green thorax and abdomen (Marshall *et al.,* 2011; Bob, 2005; Whitworth, 2006; Whiteworth, 2010; Anthony, 2011; Al-Shareef, 2016; Fahd and Sureshchandra, 2017). There are also other some uncommon, nonmetallic species of Calliphoridae subfamily; Melanomyinae which have a bare arista antenna. The blow fly families that are very important to forensic entomology include a variety of carrion feeding species such as Calliphorinae, Luciinae, Chrysominae and *Phormia* (Williams and Martin, 2014). Other examples of families Calliphoridae are *Protocalliphora* and *Trypocalliphora* but are not important in forensic studies. They are bird nest parasites that are major concern for birders and ornithologists. Some species of blow flies invade live animal

tissues causing myiasis and may be referred to as screwworm flies (*Chrysomyia*) and new world screwworm flies (*Cochliomyia*). Examples of Calliphoridae are genus *Calliphora* (*Calliphora vicina, Calliphora hilli, Calliphora stygia*), *Lucilia* (*Lucilia sericata, Lucilia cuprina*), *Pollenia* (*Pollenia advena, Pollenia antipodea*), Chrysomyinae (*Chrysomya megacephala, Chrysomyia rufifacies, Chrysomya nigripes, Chrysomya fumosa, Chrysomya marginalis*), *Hemipyrellia* (*H. ligurriens*), *Ptilonesia* (*P. auronotata*), *Xenocalliphora* (*Xenocalliphora flavipes, Xenocalliphora antipodea*) (Dear, 1986). Distinguishing features of the subfamilies include: the thorax and abdomen of luciinea and chrysomynae have brightly metallic green, purple, blue, or bronze colour while Calliphorinae have a dull grey or black thorax and metallic purple or blue abdomen. The subfamily Chrysomynae can be distinguished from the luciinae by having row of hairs above the stem-vein at the base of the wings, greater ampulla with thick hairs, as well as densely haired lower calypter and by having dark transverse bands on the dorsum of the third and fourth abdominal segments and sharply bent wing vein. The luciinae (fig. 3) are mostly greenbottle flies with bare stem-vein at the base of the wings (Whitworth, 2006; Anthony, 2011; Elleboudy *et al.,* 2016). Luciinae is one of the earliest blow flies that are attracted on a crime scene and also form maggot masses especially on the open wounds. However, *Calliphora vicina* is one of the most forensic important species of the genus *Calliphora*. They are mainly used in determination of PMI as they are the first insects that visit the crime scene. The head is dichoptic in female 3× as wide as anterior ocellus in male, 1.25× an eye width in female. They are dark-brown except on jowls, interfacial membrane, and facial ridges, which are orange in colour. Interfrontalia dusted silvery-grey to brassy, with numerous fine, black setulae; female with irregular, shifting patches centrally. Parafacialia dusted silvery to brassy, with numerous fine setulae. Jowls golden-dusted anteriorly, silvery-grey posteriorly. Face with a thin, grey dusting. Occiput grey-dusted, with creamy hairs centrally. Vibrissae strong, crossed, with 1 or 2 pairs of stronger setae. Facial ridge with short, stout setae for two-thirds of its length. Palpi orange-yellow, flattened and slightly dilated apically (Dear, 1986).

Thorax: It is dark-brown, with silvery-grey dusting. Mesonotum with indistinct, undusted vittae between setal rows. Pleurotergite with pale pile. Anterior spiracle orange and posterior one brown. Scutellum with a pair each of apical and discal setae and 2 pairs of lateral and basal setae.

Wings: The veins are dark brown. Epaulet brown; basicosta orange. Squamae infuscated brown; lower lobe with a broad, white margin and long hairs dorsally. Marginal hairs white on upper lobe, brown on lower lobe.

Legs: the legs are blackish brown. Femora and coxae thinly grey-dusted. Fore tibia with a row of short setae and middle tibia with a row of setae containing 1 or 2 stronger setae.

Abdomen: The abdomen is ground black, with metallic blue reflections and a dense, tessellate silvery dusting (Dear, 1986).

Life cycle of Calliphoridae

The life cycle (Fig 2.) of blow flies is short, and is similar in most species. Females oviposit in medium, usually carrion, producing clusters of eggs which hatch within 24 hours. Callophoridae egg is white and slightly elongated, slightly resembling a grain of rice in size, shape and colour. There are three larval instars, lasting in total of about 8 days. The stages of the larvae are identified by the position of spiracle on the body. Larvae feed on liquefied tissues which have been dissolved partly by proteolytic bacteria and partly by enzymes they secrete themselves. When they mature, the third instar larva ceases feeding and wanders away from the food supply to a suitable pupation site- generally dry and under the decomposing carcass or slightly away from it, about 3 cm below the soil surface. It empties its gut, acquires fat deposits, and contracts to form a barrel-shaped puparium (Dear, 1986). Within this structure the larva moults into a pupa, from which the adult fly emerges, usually within 2 weeks. The adult size ranges from 6 mm to 10 mm, but differs between species (Marc, 2015). Adult females have a pre-ovipositon period lasting 4-7 days, and in a total lifespan of 2-3 weeks lay 600 -800 eggs, depending on the species. At 25 °C on meat, the total life cycle from egg to egg takes 20 – 25 days. All stages are influenced by temperature, humidity, photoperiod, and the type of food available.

Sarcophagidae

The sarcophagids (fig. 3) are commonly called flesh flies. Many are scavengers and feed on decaying animal tissue, some are parasites of other invertebrates and a few parasitize vertebrates and are considered primary myiasis producers (Gillott, 1995). Carrion can attract several different species of sarcophagids depending on the time of the year and geographical area (Blackith and Blackith, 1990). Examples of the species are *Sarcophaga carnaria* and *Sarcophaga nodoso*. A few species lay their eggs in the open wounds of mammals hence, their

common name. Flesh flies differ from the tachinid flies in that they lack the postscutellum, the large swelling underneath the scutellum on the thorax. Flesh flies have a prominent row of bristles (setae) on each side of the thorax just above the base of the hind leg in addition to another row of bristles differentiate the flesh flies from the muscid flies: they rarely have both sets. They most resemble blow flies but are never metallic coloured. They generally have highly contrasting black and grey stripes on the thorax, as well as a checkered pattern on the abdomen. Sarcophagids are easily identified to family in the field, but the examination of male genitalia (hypopygia) is the only method for identification to the genus or species level, with exception of one species (Aspoas, 1994). Females cannot be identified unless they are gravid and the eggs are reared to produce some adult males for dissection (Aspoas, 1994).

Figure 1: Blue bottle fly (*Calliphora vicina*)

Source: Kniphofia (2008)

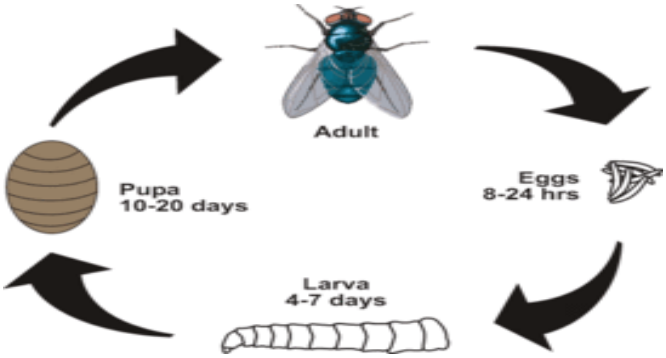


Figure 2: Life cycle of blow fly
Source: Anderson and Cervenka (2009)

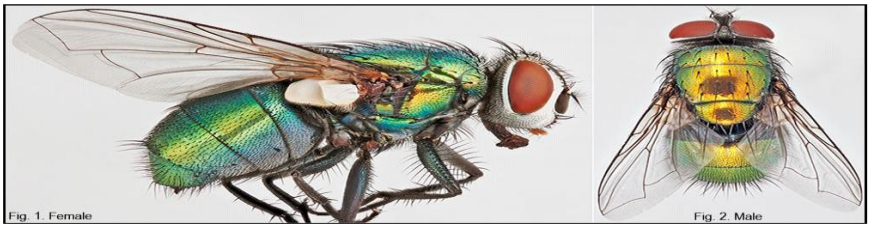


Figure 3: *Lucilia sericata* (male and female species)

Source: Anthony (2011)

Figure 4. Flesh fly (*Sarcophaga carnaria*)

Source: Fedora (2014)

Muscidae

Muscids are very common flies in a variety of habitats and some are strongly associated with human environments (homes). Species in this family are found breeding and feeding on refuse, excrement and carrion. Muscids are more commonly associated with carrion during the decay and post-decay stages, when the internal organs are exposed and this is because of their food preference. Many researchers record their presence and/or abundance, but find this to be too variable to be used as an indicator for time of death. A good example of Muscidae is *Musca domestica* (common house fly) (Ginger, 2005).

Piophilidae

Piophilids are scavengers and some are considered serious pests of cheese and preserved meats. Many larvae also feed and develop on carrion. *Piophila casei* (L.) is commonly known as the cheese skipper and is named for the jumping movement of the larvae. This characteristic movement makes it easy to recognize these larvae in the field. It is the presence of the established larval colonies that is important in terms of the succession of these flies. Adults of *Stearibia nigriceps* Meigen and *Prochyliza xanthostoma* Walker can be found in and around carrion early in the decay process but larval colonization is not evident until the advanced decay stage. The larvae feed and develop in the thick, pasty areas of the carcass and are also found in materials near the ground. Since larval piophilids carry out their development during this later stage of decomposition and are the dominant species at this time, they are used as an indicator of time of death (Ginger, 2005).

Dermestidae

Dermestidae belong to the family Coleoptera and are attracted to carcasses at specific stages of decomposition (Reema *et al.*, 2015). Dermestids feed on various things like animal's decomposing remains and skin etc. Dermestids are elongate broadly oval, covered with hairs or scales, with short, clubbed antennae fitted in grooves and of black or brownish. The combined of post bloated cadaver and male pheromones of dermestid species act as attractant for virgin females. The adult males and females copulate multiple times and females lay eggs after 24 hours of first mating. At initial stage, the larvae are creamy white but after few hours they get darken to light grey colour (Hoermann *et al*., 2011). Beetle larvae recovered from corpses can be easily differentiated from maggots as they have 3 pairs of legs and the maggots found on

decomposing remains will not have any legs. The bodies of beetle larvae may range from almost white, robust, and hairless to dark brown, slender, and quite hairy. Others may appear almost black and have armored plates on their back. These larvae can be used in investigative forensic entomology to aid in establishing a time of colonization or post mortem interval (PMI) (Razzaq, 2015). The appearance of *Dermestes maculatus* on decomposing remains of human and other animals makes it a candidate to estimate postmortem interval in cases of suicide, homicide, or unattended death. Adults generally arrive five to 11 days following death (Richardson and Goff 2001). This beetle can become one of the dominant insects present in mid to late decay. Larvae of *Dermestes maculatus* have been collected as late as day 51 following death (Richardson and Goff 2001). While the adults have been detected at early stages of decay, the larvae are the life stage used for post-mortem interval estimation. Larvae do not appear on corpses until the later stages of decay when the body has dried out. Full development of *Dermestes maculatus* is only reached when temperatures are consistently above 18°C, and will take 96 days at 18°C from the time the egg is laid to reach adulthood (Arnaldos *et al.*, 2004). The optimum temperature for *Dermestes maculatus* development is approximately 30°C, where the beetles reach adulthood around 38 days (Richardson and Goff, 2001).

Staphylinidae

Staphylinids are the most common predators on carrion and prey on many insects, but seem to feed heavily on maggots. More than fifty species have been identified on pig carrion with the first arriving in the early bloat stage and remaining until all insect activity ceased. Both larval and adult staphylinids are predators and many species of larval Aleocharinae are ectoparasites of calliphorid pupae (Ginger, 2005).

Predators and Parasites of Necrophagous Species

The predators and parasites of the necrophagous species comprise the second most significant group of carrion-frequenting taxa. Many of the beetles (Coleoptera: Silphidae, Staphylinidae, and Histeridae), true flies (Diptera: Calliphoridae, Muscidae and Stratiomyidae), and Wasps (Hymenoptera) are parasitic on fly larvae and pupae. In some species, fly larvae (maggots) that are necrophagous during the early stages of their development become predators on other larvae during the later part of their development, as is the case for *Chrysomya rufufacies.* The larvae of

this species are both predatory and cannibalistic. They often consume the larvae of other fly species (Jens *et al.*, 2010).

- **Adventive Species**

This category includes those arthropods that simply use the corpse as an extension of their own normal habitat. Examples of adventives species are springtails (Collembola), spiders, centipedes, millipedes, grasshoppers, crickets, mites and pill bugs (Ginger, 2005; Jens *et al.*, 2010).

- **Accidental Species**

Another category that is not always recognized but may still be of significance is what might be termed "accidentals." These are species that have no real relationship to the corpse but still are found on the body. These insects may have fallen onto the body from surrounding vegetation, thus possibly useful in determining the postmortem movement of a body. On the other hand, when an insect stops flying, it has to land on something and that "something" might happen to be the body, with moths, butterflies, ants and bees serving as examples. It should also be noted that many spiders, centipedes and ants are found in and around carrion and may be considered incidental predators (Ginger, 2005).

1.2.2 **Decompositional stages of carcass**

Decomposition of an exposed cadaver is a continuous process, beginning at the moment of death and ending when the body is reduced to a dried skeleton (Mohammed, 2015). Decomposition is a natural and necessary process responsible for the return of organic material, such as dead plant or animal matter, to the ecosystem. Arthropods that feed on dead vertebrate bodies including man and other animals (carrion feeder) make them significantly important in forensic science. By their feeding action, they form part of the natural recycling of organic matter in the ecosystem in which they are found. The phases of decomposition are made up of the early and late postmortem changes including putrefaction, decay and skeletonization (James, 2009).

Early stages of decomposition

Early changes of postmortem interval include the gradual changes in the physical nature and /or appearance of the body prior to the onset of gross and recognizable decompositional changes. These changes have traditionally been used in estimations of the PMI and may be a source of confusion if not recognized. The early stages of decomposition include:

Livor mortis: One of the early changes observable on a decomposing body is livor mortis, also referred to as lividity, postmortem hypostasis, vibices and suggilations. This is a physical process. While the individual is alive, the heart is functioning and circulating the blood. When death occurs, circulation stops and the blood begins to settle, by gravity, to the lowest portions of the body. This results in a discoloration of those lower, dependent parts of the body. Although beginning immediately, the first signs of livor mortis are typically observed after a period of approximately 1 hour following death with full development being observed 2–4 hours following death. At this time, the blood is still liquid and pressing on the skin will result in the blood being squeezed out of the area (blanching), only to return once pressure is removed. This situation continues until 9–12 hours following death, at which time the pattern will not change and the livor mortis is said to be 'fixed.' Any areas of pressure resulting from clothing or continued pressure during this period will not show discoloration (Goff, 2009a; Mohammed, 2015).

Rigor mortis: This is a chemical change resulting in a stiffening of the body muscles following death due to changes in the myofibrils of the muscle tissues. Immediately following death, the body becomes limp and is easily flexed. As adenosine triphosphate (ATP) is converted to adenosine diphosphate (ADP) and lactic acid is produced lowering the cellular pH, locking chemical bridges are formed between actin and myosin resulting in formation of rigor. Typically, the onset of rigor is first observed 2–6 hours following death and develops over the first 12 hours. The onset begins with the muscles of the face and then spreads to all of the muscles of the body over a period of the next 4–6 hours. Rigor typically lasts from 24 to 84 hours, after which the muscles begin to once again relax. The onset and duration of rigor mortis is governed by two primary factors: temperature and the metabolic state of the body. Lower ambient temperatures tend to accelerate the onset of rigor and prolong its duration, whereas the opposite is found in warmer temperatures. If the individual has been involved in vigorous activity immediately prior to death, the onset of rigor is more rapid. Body mass and rates of cooling following death also influence the onset and duration of rigor mortis. As rigor disappears from the body, the pattern is similar to that seen during the onset, with the muscles of the face relaxing first (Goff, 2009b).

Algor mortis: Once death has occurred, the body ceases to regulate its internal temperature and the internal temperature begins to approximate the ambient temperature. In most instances this involves a cooling of the body until ambient temperature is reached, most often in a period of 18–20 hours. Any estimate of the post-mortem interval obtained using this technique should be

limited to the very early stages of death (18 hours or less) and treated with care. There are several obvious factors involved in the cooling of the body that may easily influence the rate at which this occurs. The size of the individual is a major factor. A smaller individual will cool more rapidly than a larger individual in the same set of conditions. Exposure to sunlight or heating may also influence the rate of cooling as many clothing and a number of other factors. The most commonly used temperature in these calculations is from the liver although rectal temperature may also be employed (Mohammed, 2015).

Greenish discoloration: As the body decomposes, gases are produced in the abdomen and other parts of the body. Although the exact composition of the gases may vary from body to body, a significant component of these gases is hydrogen sulphide (H_2S), a small molecule that readily diffuses through the body. Hydrogen sulphide will react with the haemoglobin in blood to form sulfhaemoglobin. This pigment is greenish and may be seen in blood vessels and in other areas of the body, particularly where livor mortis has formed. Other gases are hydrogen, ammonia and sulphur dioxide

Skin slippage: The outer layer of skin, stratum corneum, is dead. It plays a vital role in water conservation and protection of the underlying (live) skin. This layer is constantly being shed and replaced by underlying epidermis. Upon death, in moist or wet habitats, epidermis begins to separate from the underlying dermis due to production of hydrolytic enzymes from cells at the junction between the two layers. The epidermis can then easily be removed from the body. Slippage may first be observed as the formation of vesicle in some dependent parts of the body. In some instances, the skin from the hand may separate from the underlying dermis as a complete or relatively complete unit. This is termed ‘glove formation’ and can be removed from the hand as an intact unit. This skin can be used for finger printing, often with better results than if the skin remains on the hand (Mohammed, 2015).

Tache noir: Following death, the eyes may remain open and the exposed part of the cornea will dry, leaving a red-orange to black disation. This is termed ‘tache noire’ (French for ‘black line’) and may be misinterpreted as haemorrhage. Unlike haemorrhage, this will have symmetrical distribution, corresponding to the position of the eyelids.

Marbling: As the anaerobic bacteria from the abdomen invade the blood vessels, the subcutaneous vessels take on a purple to greenish disation. These take on a mosaic appearance,

similar to what is seen in cracking of old marble statuary. Typically this is seen on the trunk and extremities (Goff, 2009a).

Mummification: In a dry climate, portions of a body or the entire body, having a large surface area to mass ratio, will desiccate. The low level of humidity will serve to inhibit bacterial action and typically there will be some exclusion of insects and other scavengers from the body. The temperatures will be either very high or very low in this type of situation. The desiccated tissues and skin will have a leathery appearance and will survive for long periods of time with minimal change. In hot, dry climates, mummification can occur within a period of several weeks (Rao, 2013).

Saponification: This is the process of hydrolysis of fatty tissues in wet, anaerobic situations, such as submersion or in flooded burials. The tissues take on a waxy appearance and consistency. This process requires a period of several months to complete.

Later stages of decomposition

Forensic entomology divided later phases of decomposition into five stages based on the physical appearance of the carcasses; fresh, bloated, active decay, advance-decay and remain (skeletal) stage (Anderson and Cervenka, 2009; Goff, 2009a; James, 2009; Feugang *et al.*, 2011; Isaac *et al.,* 2011; Muhammad *et al.,* 2013; Farzana and Anzela, 2013; Sundharavalli, 2014; Robin and Nor, 2015). The stages are;

Fresh stage (day 1 - 2): This begins at the moment of death and ends when the bloating of the carcass is observed. Although autolysis occurs at this stage but gross morphological changes do not occur at this point. Insects are usually attracted within the first 10 minutes of death to the carcass and they lay eggs (oviposition) as soon they arrive. Even though morphological changes are not obvious to humans, chemicals released from the cellular breakdown attract insects in this early stage (Isaac *et al.,* 2011).

Bloated stage (day 2 - 7): Putrefaction begins at this stage. Gases produced by the metabolic activities of anaerobic bacteria cause an inflation of the abdomen and the carcass forming a balloon-like appearance during the later part (Amendt *et al.,* 2011). Arthropod activities combined with the putrefaction processes cause internal temperatures of the carcass to rise. The greatest numbers of adult Diptera are attracted to the carcasses during this stage.

Active decay stage (days 5 - 13): Abdominal wall is penetrated, resulting in the deflation of the carcass and ending the bloated stage, the internal temperature rises to 14 °C above the ambient temperature followed by a drop signifying the end of the decay stage. Decay is high during increasing temperatures and drops with a fall in temperature. There is a steady decrease in the weight of the carcass by 10^{th} day. There is a conversion of carcass biomass to diptera larval biomass. The larvae subsequently depart from the carcass to pupate.

Advance-decay stage (days 10 - 23): The post-decay stage begins when most of the Diptera larvae leave the carcass, leaving behind bones, cartilage, hair, small portions of tissue, and a large amount of wet, viscous material known as byproducts of decay (BOD). The BOD is the major site of arthropod activity during this stage.

Dry skeletal remains stage (days 18 – 90+): This stage is characterized by bones with little cartilage remaining and the BOD has dried up. The transition from post-decay to remains stage is gradual, with declining adult and larval dipteral population (Isaac *et al.,* 2011; Bala and Kaur, 2015).

A study on buried pork in India noted that the first stage of development started between 0 – 3 days of experiment and no odour was detected during the stage. The bloated stage started between the 4^{th} – 8^{th} days. Odour of decay was noticed during this stage and fluid started seeping from the pork. Decay stage started on 9^{th} to 13^{th} days and the pork was deflated and most of the parts were relatively dry (Bala, and Kaur, 2015). In the study of Coleoptera species associated with dog (*Canis domesticus* L.) cadaver, it was observed that fresh stage lasted for 0 - 12 hours after death. The outside appearance of the bodies was similar to those of live dogs but the body emitted very strong smell that was higher than the first day. Active decay comprised the third stage, the skin of the *C. domesticus* appeared black, emitted less smell and 80-90 % of the body was decomposed. The total duration of this stage was 3-4 days after death (49 - 96 hours). Advanced decay Stage was 5-6 days after death (97-144 hours) and was characterized by minor deep and the removal of the soft internal tissues. The body was decomposed up to 90-99 %. Dry stage took five days 7-11 (145-265 hours.) of decomposition (Muhammad *et al.,* 2013). Prado *et al.* (2011) studied the activities of blow flies using pig cadaver observed that the fresh stage began at the moment of death and lasted until day 2, both in sunny and shaded sites. On day 3, bloating was evident in the carcass exposed to the sun, while in the one in the shade only a slight

bloat was noticeable. This indicated that putrefaction had begun, so in both sites bloated stage was considered to begin on the 3rd day. The carcass in the sun was very inflated on day 4 and was very strong, whereas the carcass in the shade only on day 6 was with equivalent aspect and was never intense as in the sun. Active decay stage started on day 7. Dipterous larvae began to migrate massively from the carcasses on day 8 in both sites, marking the beginning of advanced decay stage. The migration lasted approximately one week, with the number of larvae collected being higher in the shade. Dry stage was reached on day 15 in the carcass exposed to the sun, and on day 42 in the shaded carcass, although the frequent rains rehydrated the carcasses, attracting more flies. According to the work of Abajue and Ewuim (2015) who observed four stages of decomposition (fresh, bloating, active and dry decay), fresh stage lasted between 0 – 12 hours and bloating lasted between day 2 -4. Active stage lasted through day 5- 8, while the dry stage lasted from day 9 – 16. Ahmad *et al.* (2011), Observed five stages of decomposition which include; fresh, bloated, active decay, post decay and remains stage. In the outdoor carcass, fresh decomposition stage lasted between Day 1-2 and bloated stage lasting 3 days (Day 3 – 5). The decay stage lasted for 2 days (Day 6 - 7), during which many maggot masses were seen in the mouth, The advanced decay stage (Day 8-9) had lesser odour with many maggots pupated under the carcasses. They also observed that decomposition lasted longer in the indoor carcass than in the exposed carcass and there was also a delay of fly arrival for at least 3 days for both the replicates.

Post Mortem interval (PMI)

Post mortem interval is the length of time between death and corpse discovery (Sukontason *et al*., 2005; Ruchi *et al.*, 2015). In forensic science, the use of maggots helps when determining the post mortem interval (PMI). According to Ruchi *et al.* (2015), determination of the post mortem interval is a crucial and fundamental step in any death scene investigation when the death has not been witnessed. An accurate estimation of time since death (PMI) is an important aspect of every death investigation (especially in suspicious deaths) particularly for highly decomposed or skeletonised bodies, to reconstruct the events and circumstances of death, to link a suspect to the victim and to establish the credibility of statements made by the expert witnesses (Jagmahender and Sharma, 2008). At the onset of death, the medical parameters can help to establish the cause, manner, and time since death. As soon as death occurs, the cells, tissues and organs of the body begin to degrade with the progression of time. A post mortem determination by a pathologist or

medical examiner becomes more difficult and less accurate when it has passed fresh states of decomposition. Forensic entomology is now an integral part of death investigation when estimating the time since death beyond 72 hours. Forensic entomology is considered the most accurate method for estimating the elapsed time since death, particularly when more than 3 days have elapsed. The PMI includes initial egg laying and time of maggot development until maggots are collected at the crime scene (Gennard, 2012). Blow fly maggots usually feed upon carrion. As soon as they invade the cadaver, the female flies of these necrophagous species start laying their eggs in open orifices of the body (eyes, ears, mouth, nose, vagina, anus, or wounds). After eggs hatch, maggots begin to feed on the carrion, eventually forming a maggot mass, and once feeding is finished (in the middle of the third larval stage) usually migrate off the carrion to pupate and eventually emerge as adult flies.

Determining the PMI is not a simple calculation as it includes calculating the time it takes for blow flies (in many cases) to complete its life cycle. For an accurate analysis, scene temperatures must be calibrated to available recorded temperatures, potential environmental factors (e.g., rain) or conditions of a body (e.g., clothing, burning) must be considered (Sharma *et al.,* 2013). It is also known that maggot masses cause an increase in temperature. Maggot masses increasing the temperature of the carcass will also affect the development of these maggots as increase in temperature is directly proportional to insect development (Amendt *et al.,* 2011). Furthermore, researchers have not examined oxygen concentrations of these masses and how this lack of oxygen affects the maggots in the mass. It is possible that the competition to feed may be affected by both the temperature and the oxygen levels of the mass. The estimation of PMI is based on few valid assumptions, and errors in any of these assumptions can lead to wrong estimates of time since death. Investigations should not be completely dependent on the few baseline studies. This means that data generated in one area of study should not be used to determine the PMI in a different region. Therefore, database should be developed in every region, in which insects are being used to determine the time of death (Kapil and Reject, 2016). Furthermore, PMI can also be affected by period of insect activity. Most insects are aquatic, terrestrial, diurnal, nocturnal, seasonal, while some are restricted to shade or sunny environment. These factors can affect the rate and type of species that will colonize a decomposing body. For instance when death occurs in the night, the first insects that may likely visit the scene are nocturnal insects because they have the highest period of activity in the night. A similar analogy

was the observation made by Ubachukwu (2005) where it was noted that *Simulium* flies are diurnal and their biting activity stops at dusk.

1.2.4 Methods of estimating post mortem interval

There are two methods of estimating time since death:

Maggot age and development: Maggot development is used when death occurred less than a month prior to discovery. Maggots are immature flies and Calliphoridae (blow flies) are the most common insects used. Blow flies are attracted to a corpse very soon after death and lay their eggs in natural openings or in a wound, if present. Eggs are laid in batches and hatch after a period of time into first instar (or stage) larvae. The larva feeds on the corpse and moults into a second, and then third instar larva. The size and the number of spiracles (breathing holes) determine the stage. When in the third instar, the larva stops feeding and leaves the corpse to find a safe place to pupate. This is the pre-pupal stage. The larva's skin hardens into an outer shell, or pupal case, to protect it as it metamorphoses into an adult. Freshly formed pupae are pale, but darken to a deep brown in a few hours. After a number of days, an adult fly emerges, leaving an empty pupal case behind. Each developmental stage takes a known amount of time, depending on the temperature and availability of food. Temperature is especially important since insects are 'cold-blooded' - meaning their metabolic rate increases (and the duration of development decreases) as the temperature rises, and vice-versa. Looking at the oldest stage of insect and the temperature of the region, a forensic entomologist can estimate the day or range of days in which the first insects laid eggs and provides an estimate of time of death. This method applies until the first adults emerge. After this, it is impossible to determine which generation is present and time since death must be estimated from insect succession (SFU Museum of Archaeology and Ethnology, 2010).

Using successional waves of insects: Insect succession is used if the individual has been dead for a month or longer. Insect succession uses the fact that a body (human or otherwise) supports a rapidly changing ecosystem as it decomposes. As they decay, the remains go through physical, biological and chemical changes, and different stages attract different species of insect in sequential manner. Calliphoridae (blow flies) and Sarcophagidae (flesh flies) may arrive within 24 hours of death if the season is suitable or within minutes if blood or other body fluids are

present. Other species, like Piophilidae (cheese skippers), are not interested in the fresh corpse, but are attracted to the body at a later stage of decomposition. Some insects do not seek the body directly, but arrive to feed on other insects at the scene. Many species are involved at each decomposition stage and groups of insects may overlap with each other. Knowing the regional insect fauna and times of colonization, a forensic entomologist can determine a period of time in which death took place. They may also be able to establish the season of death (*e.g.* summer) according to the presence or absence of certain insects that are only seasonally active (SFU Museum of Archaeology and Ethnology, 2010).

1.2.5 Factors affecting PMI

Many factors can affect insect succession and the decomposition of carrion. Factors such as temperature, season, time of day, accessibility and physical position of a carcass, type of death, size and type of carcass, vertebrate scavengers, insect abundance and the biology and geographical distribution of the necrophagous insects can influence the time of arrival and the duration of stay of insects on the carrion. These many factors make it necessary to study insect succession on carrion in different regions and under different conditions. Generalizations can be made regarding these factors in estimating time of death; however, it leaves open to the possibility of erroneous conclusions that are detrimental to legal cases involving murder and other serious crimes (Ginger, 2005).

1.2.5.1 Types of death

There are four manners of death that can occur. These main categories of death are what a forensic examiner will take into consideration when handling the deceased (Jack, 2016). These four categories are:

Natural death: This is simply when the body ceases to function on its own accord or if there are medical factors like terminal illness or heart disease which would bring about death.

Homicide: This is taking of one's human life by another human being by means of pre-meditated murder. The term pre-meditated murder means to have purposely planned and executed the murder of another human being in cold blood whilst trying to elude capture by the authorities. Most of the homicide death involves the presence of open wound and this could increase arthropod colonization and decomposition. Necrophagous insects normally lodge on the natural openings of the bodies such as mouth, nose, eyes, ears etc. However, when there is

presence of an open wound, insects are greatly attracted to such area as it contains blood and open flesh which it can feed on other than the natural body openings. In such carcass, the first insect colonizers will always be found in such areas and this will aid an entomologist during collection of insect evidence for determination of PMI in such carcass. The presence of open wound on the diseased could also suggest what happened before such a violent death. For example, the presence of wound on the anus attracts more insects and this could suggest that the victim was raped to death thus opening another horizon of investigation. There could be aggregation of maggots on such wounds which could increase the ambient temperature of the cadaver and accelerate the maggot development, hence leading to error PMI determination (Jack, 2016).

Accidental death: As the term would suggest, this is the death of an individual by means other than natural death, murder or suicide. Accidental death can sometimes be manslaughter-murder but committed out of an involuntary act of violence towards another. Likewise accidental death can also be categorized as death by misadventure. This means that the victim has died by accident either whilst doing or by taking risks that would put him/her in mortal danger.

Suicide: This is a deliberate taking of one's own life due to extreme emotional distress often brought about by severe depression. Suicide is neither accidental nor is classified as death by misadventure simply because the individual has set about on a course of action that would end with their own inevitable death. Normally this would occur by means of drugs overdose, the cutting of one's wrists to induce uncontrollable bleeding, or hanging oneself on a tree (Jack, 2016). The manner of death is always important when investigating the cause of death. A forensic professional will observe both physical and laboratory evidence to find out the real cause of death. Manner or cause of death can be determined using the good knowledge of insect activities especially when a pathologist can no longer get samples (blood, skin, tissue) for autopsy. The high level accumulation of these toxic substances on maggots occurs when the rate of absorption exceed the rate of assimilation (Amendt *et al.,* 2011). Various studies have shown that ante mortem use of various drugs and toxins affect maggot developmental rate, manifesting into an inaccurate PMI estimation based on insect development. Substances like cocaine, heroin, morphine, methamphetamine, methylene dioxymethamphetamine, triazolam, oxazepam, chloripriamine, barbiturates, codeine, benzoylecognine, amphetamines, tricyclic antidepressants, benzodiazepines, malathion, nortriptyline and amitryptiline can influence the

development of insects found on a carcass hence causing error PMI (Magni *et al.,* 2014). Cocaine, methamphetamine and heroin in the carcass can accelerate the larval development (Isaac *et al.,* 2011). Poisons like morphine and malathion were both believed to slow down the rate of insect colonization. Dayananda and Kiran (2013) observed that cocaine (at the lethal dose) causes larvae to "develop more rapidly (36 to 76) hours after hatching". The amount of growth depends on the concentration of cocaine in the area being fed upon. The amount of methamphetamine, on the other hand, affects the rate of pupal development. Campobasso *et al.* (2004), observed that the presence of parathion only repelled arthropods or had insecticidal effects at the mouth of the treated rabbits which contrasts the observation with malathion, where oviposition was delayed and differences were seen in the number of insect taxa found on carcasses. Errors of up to 29 hours can occur in PMI estimates with heroin containing tissues based on development of the fly *Boettcherisca peregrina*. Similar results were reported for methamphetamine and amitryptiline. Errors of up to 24 hours can occur in estimates with heroin on *Lucilia sericata* (Kapil and Reject, 2013**).** Presence of malathion in the body elongated the period of pupation in *Chrysomya megacephala*, the presence of morphine in tissues delays maggot growth.

1.2.5.2 Environmental factors

The understanding of the environmental factors inhibiting or favoring colonization, development and succession of necrophagous insects is a pre-requisite in determination of postmortem interval using entomological data (Adair, 2012). Development of flies on cadaver is affected by several factors including; temperature, humidity, burial or mummification, burning, size of the carcass, geographical region, season, maggot mass and open wounds on the body (when the carcass are man-slaughtered). Temperature is the most important environmental factors affecting the rate of arthropod development and may cause diapauses when it is extremely low (Kapil and Reject, 2016). The information on this factor is very important since they can be crucial in estimating the PMI.

Temperature

Temperature is the degree of coldness or hotness of a body. Temperature is one of the most crucial factors affecting insect colonization and development. Insects are cold-blooded organisms – the temperature of their bodies is approximately the same as that of the environment; therefore, temperature is probably the single most important environmental factor influencing insects behavior, distribution, development, survival and reproduction (Eyo *et al.*, 2014). Insects generally thrive in high temperature (15 – 37 °C) condition and can hibernate in extreme low temperature (-4 °C) (condition known as diapauses). Therefore, when the temperature is high, it could lead to rapid development of maggots and when the temperature is low, colonization, oviposition and development of maggots will be very slow hence resulting in error in postmortem interval. Kapil and Reject (2016), in their study of the effects of temperature on the developmental rate of *Lucilia sericata* and *Chrysomya megacephala* noted that *L. sericata* developed at temperatures between 22 °C and 26 °C (mean 24 °C) and relative humidity 55 % ± 10 % and *Chrysomya megacephala* at temperature between 23 °C and 27 °C (mean 25 °C) and relative humidity 55 % ± 10 %. The resultant PMI was 10 – 11 days for *L. sericata* and 8 – 9 days for *C. megacephala*. In the work of Muhammad *et al.* (2013), on decomposition of dog cadaver, noted that the average temperature (28.3±1.8- 40.4±1.7) was found to affect Coleoptera adults, larvae and rate of decomposition of *C. domesticus* during the observation period of 11 days. Prado *et al.* (2011) reported that the average monthly air temperatures measured by the nearest weather station, 8 km from the study site were 22.4 °C in shaded site and 25.3 °C in the exposed site. Zuha *et al.* (2016) observed that outdoor ambient temperatures during their research periods ranged between 26.8–33.0 °C and 25.2–29.8 °C.

1.2.6 Mock crime scene/ reconstructed crime scene: A valuable tool that is becoming very common in the training of forensic entomologists is the use of mock crime scenes (Adair, 2012). Crime scene reconstructions are quite familiar with the concept of the postmortem interval (PMI) as it is frequently used in forensic pathology. The pig or other mammal carcass represents a human body model and can be used to illustrate various environmental effects on both arthropod succession and the estimate of the post mortem interval (Adair, 2012). These animal models are used because most mammals have the same decomposition rate as that of humans and also human cadavers are not always available in such a study (Ekrakene and IIoba, 2011; Prado *et al.*, 2011). Mock crime scene can also be used to observe the stages of decomposition of carcass and

generate data for calculation of PMI using succession of series of flies that enhance decomposition. A postmortem interval estimate is an important component of any crime scene reconstruction. It provides a useful timeline from which all other evidence may be evaluated. Certainly there are times when a lack of data complicates efforts to fully understand the forensic time line, but failure to recognize evidence which may affect the entomological time line creates potential inaccuracies that may prevent a proper resolution of the case (Adair, 2012). However, since the cause and manner of death can influence postmortem processes, it is customary to study decomposition and succession using a variety of scenarios that mimic known crime scenes. Animal as a model in a mock death scene could be made to die in different ways such as narcotizing them with toxic chemicals, burning, killing with sharp objects, preserving them after death (with pesticides), burial (Marcin and Krzysztof, 2016), to generate data for studying the post mortem interval and determination of how, where, and the possible chemical that caused such death on any type of death crime cases. This will serve as a valid reference in the court where forensic entomology is practiced. If, as an example, one wishes to test the effect of restricting insect accessibility by use of a shelter, then a typical experimental design could involve one carcass exposed inside a shelter and another carcass exposed outside directly on the ground (Michaud, 2012). Entomologists are not expected to incorporate this data if it is not well furnished on a reconstructed crime scene (Adair, 2012).

1.2.7 Criteria for observation and collection of forensic specimens

Forensic investigations rely on evidence and material found at a crime scene, which must be recorded and collected carefully. This is especially true for insect material, which can be hard to find. When approaching a scene to collect insect evidence, a forensic entomologist first considers the surroundings. If the scene is outdoors, they note the landscape, plants and soil types, as well as the weather (SFU Museum of Archaeology and Ethnology, 2010). The following considerations should be properly made before collection of samples from the crime scene:

Is the carcass on woods, a beach, a house, a roadside? Vegetation: trees, grass, bush, shrubs?

Soil type: rocky, sandy, muddy? Elevation and map coordinates of the death site Is the site in shade or direct sunlight? Presence, extent and type of clothing? Is the body buried or covered, if so, how deep and with what (soil, leaves, cloth)? What is the cause of death, if known, in

particular, is there blood at the scene, or other body fluids? Are there any wounds, if so, what kind? Are drugs likely to be involved? This may affect the decomposition rates. What position is the body in? What direction is the body facing? What is the state of decomposition? Is maggot mass present, how many? This will affect the temperature on the body. What is the temperature of the centre of the maggot mass(s)? Is there any other meat or carrion around that might also attract insects? Is there a possibility that death did not occur at the present site (SFU Museum of Archaeology and Ethnology, 2010).

The procedure for the forensic entomological analysis or crime scene includes; Visual observations and notations: These include observation of type of habitat, the number and kinds of insect species, resting and crawling insects, major infestations associated with the body and the surroundings, insect predators, exact position of the body, insect activity within 3-6 m of the body, any unusual, naturally occurring or man-made phenomenon altering the body.

1.2.8 Collection of insect evidence at a crime scene. Collection of climatological data: As the length of insect life cycle is influenced by temperature, precipitation and humidity of the environment, climatological data in estimating PMI is quite crucial. Ambient, ground, body-surface, under-body interface, maggot mass and soil temperatures are procedurally noted. Weather data of scene (when victim was last seen up to discovery of body) may be collected from the nearest meteorological station. The distance between the death site and the weather station is also important to be considered to avoid much variation in climatic data.

Collection of specimens from the body and the surrounding area (up to 6m from the body) before removal of remains: The natural orifices, traumatic wounds of the cadavers and eyes are the preferred sites of oviposition by the insects. The other places of interest are corpse-substrate interface, under the body, in the pleats of clothes and pockets, shoes of the deceased and on the carpet or bag in which the body might have been wrapped for storage or for transportation.

Collection of specimens from directly under and in close proximity (1 meter or less) after removal of remains: Entomological specimens should be collected from the area surrounding the body before its removal and also from directly under and in close proximity to the remains after removal. Intensive search of the area up to at least 2 meters away from the body is required for the insects that might have dispersed from the body for pupation (Jagmahender and Sharma, 2008).

However, insect evident are collected in the following manner; adult insects are collected using sweep net by sweeping around the carcasses to catch the flying insects (Ahmad *et al.,* 2011; Khoo, 2012; Parmod, 2012; Bala, and Kaur, 2015; Szymon *et al.,* 2016). For maggot and other immature insects, specimens are collected using forceps or fine arts' brush (Ahmad *et al.,* 2011; Parmod, 2012; Muhammad *et al.,* 2013; Abajue and Ewuim, 2016; Szymon *et al.*, 2016). Pitfalls are made around the carcass in other to catch the crawling insects and the third stage larvae which are migrating to their pupating sites (Szymon *et al.,* 2016). Hanadi and Mesbah (2010) used a sticky trap positioned half a meter from each caged carcass. At each visit, two caged carcasses, one from each group, with the corresponding sticky traps were removed and placed in large plastic bags which were closed and transferred to the laboratory for entomological examinations. Prado *et al.* (2011), in their work used two modified Schoenly (1981) traps, one placed in the sunny area and the other in the shady one. The traps are dodecahedral structures constructed in plywood. The base of the traps is a large mesh plastic net, so the carcasses were in direct contact with soil; the top of the traps is covered with fine mesh plastic net, which allows good aeration and odour dissipation. Each trap was baited with one freshly killed piglet. A 40 % ethylene glycol solution with formalin and detergent was used in the trap as killing and temporary preservative agent for the arthropods. Ekrakene and Iioba (2011), in their work used sweep net to collect the flying insects while other crawling insects were done by carefully examining debris around the deposited pigs from 30 – 50 cm from the carcasses. Vanin *et al.* (2013) used eight insect pitfall traps containing a saturated NaCl solution

and soap which were placed at 50 cm all around the carcass. They also use hand picking in collection of samples on the carcasses, under them, and where and when possible in the carrion cavities. Ruchi *et al*. (2015) used sweep net and pitfall trap for their collections. Szymon *et al.* (2016), in their work used two pitfall traps (plastic containers 16 cm in diameter and 17 cm in height) per carcass. Traps were filled with 50 % ethylene glycol solution and were emptied at every inspection. Abajue and Ewuim (2016) used blunt forceps or fine arts' brush and sweep net during their collections.

1.2.9 Rearing and preservation of the collected samples

An excellent technique for preservation is to blanch the larvae in hot (nearly boiling) water for 60–120 s, and then place the blanched larvae in 80 % ethyl alcohol. An alternative is to place the collected eggs or larvae directly into a preservative solution. The suggested preservative fluid is KAA (95 % ethanol, 80–100 ml; glacial acetic acid, 20 ml; and kerosene, 10 ml) (Goff, 2009b). However, it is important to understand that with soft-bodied insect larvae, a simple placement of the insect directly in 80 % ethyl alcohol is not an adequate method of preservation because the chemicals shrinks and bleaches it hence difficulty in identification using morphological characteristics (Ekrakene and IIoba, 2011; Khoo, 2012; Ruchi *et al*., 2015). The collected material in the form of eggs, larvae or pupae are reared by transferring them to transparent glass beaker partially filled with sterilized sand covered with circular piece of filter paper (Parmod, 2012) or sawdust (SFU Museum of Archaeology and Ethnology, 2010). A piece of flesh from same body is provided as food for the development of the insect samples to adult stage. After transferring immature stage insects the mouth of jar is covered with muslin keep in position with rubber bands. The glass beakers are observed daily for emergence of adults and development period are noted. Temperature is especially important and if possible, a portable recording device is left to record long term changes. Abajue and Ewuim (2016) used general purpose 'glass rod' mercury thermometer and portable digital thermo hygrometer to measure daily ambient temperature and relative humidity of the study area respectively. When the adults emerge, they are placed in vials with 70 % alcohol and later pinned and identified (Ahmad *et al.,* 2011; Parmod, 2012; Szymon *et al.,* 2016).

1.2.9 Insects arrival on the scene/ insect succession, composition and relative abundance

Calliphoridae being the most important forensic family are the first insects to arrive on crime scene. They arrive some minutes after death, suggesting that they have high sense of smell to detect a decaying body within minutes thus useful in the calculation of PMI between days to one month of death (Jadav and Sathe, 2015). Blow flies have forensic value and are very useful for solving problems related to murder, suicide, sexual molestation, child neglect and abuse etc. (Sukontason *et al***.,** 2005; Jagmahender and Sharma, 2008; Jens *et al.,* 2008). However insect colonization of carrion can be described as a rapid invasion of the carcass by adult calliphorids, sarcophagids and muscids, resulting in the presence of huge numbers of Diptera eggs and larvae. This provides an abundant food supply for predacious beetles such as silphids and staphylinids. Insect diversity reaches a maximum during the fresh, bloat and decay stages of decomposition. As the carcass decays, there is a distinct decrease in species richness. As the food resource becomes depleted, insects disperse from the carcass and different species that prefer the later stages of decay for food and development arrive. Piophilids, clerids and nitidulids are typically associated with the carcass during the advanced decay stage and dermestids are typically associated with the dry remains stage. This change in species composition on carrion over time is called insect succession (Ginger, 2005). Michaud *et al.* (2012), in their study using 7 domestic pigs (*Sus scrofa*) observed a total of 130 necrophagous and predacious insect species representing 2 orders, 18 families and 75 genera throughout their study. The Diptera were represented by 7 families and 34 species and Coleoptera were represented by 11 families and 96 species, with 38 % of the species being considered as adventive species. Bala, and Kaur (2015) reported the successional studies of 5kg of clothed pig carcass which was buried at a depth of 30cm in the forest area of Ghawaddi village of Ludhiana (Punjab) (India). The pork was exhumed two times, morning and evening. The whole process of decomposition took sixteen days and 10 beetle species belonging to 6 families were observed. They also observed 2 orders of Hymenoptera (*Camponotus compressus* and *Pheidole indica)* belonging to family Formicidae during different stages of decomposition. In the work of Prado *et al.* (2011), they observed a total of 38594 adult Diptera during the 121 days of the experiment, including 12033 captured in the sunny site and 26561 collected in the shaded one. Of these, 10723 belong to the Calliphoridae family, with 6261 individuals collected in the sun and 4462 in the shade. Eleven

calliphorid species were identified: *Calliphora vicina*; *Calliphora vomitoria, Chrysomya albiceps*; *Lucilia ampullacea*; *Lucilia caesar*, *Lucilia illustris, Lucilia sericata, Lucilia silvarum, Pollenia sp., Protophormia terraenovae,* and *Stomorhina lunata*. All the species were present both in sunny and shaded sites. Higher numbers of specimen were collected in the sunny site than in the shaded one, particularly those from *C. albiceps.* The exception was *C. vicina*, which was slightly more abundant in the shade. *Calliphora vomitoria* arrived to the corpse on the first day in the shaded site and second day in the sunny site. Reema *et al.* (2015) studied succession and life cycle of beetles using 2 pig carcasses weighing10 kg. They observed that necrophagous insects mainly Coleoptera are attracted to carcasses at specific stages of decomposition. Males of *Dermestes maculatus* were seen on the cadaver after 9-10 days but larvae infestation occurred after15 to 21 days. The adult males and females copulated multiple times and females lay eggs after 24 hours of first mating. Robin and Nor (2015) used two decomposing rabbits (*Oryctolagus cuniculus*) carcasses to study forensically important flies associated with decomposing carcasses. They reported that a total of 229 and 219 individual species were collected in rabbit carcasses throughout the decomposition process at Kolej Kenanga in UNIMAS, East Campus, Kota Samarahan and Jalan Bako, Kuching respectively. In all sites three Orders were present namely Diptera, Coleoptera and Hymenoptera. Major species of insect identified belonged to the genus Chrysomya represented by *Chrysomya megacephala, Chrysomya rufifacies, Chrysomya nigripes* and *Chrysomya villeneuvi*. Three other blow flies species recorded were *Hemipyrellia ligurriens*, *Lucilia cuprina* and *Hypopygiopsis violacea*. The study also revealed domination of *Chrysomya megacephala* and *Chrysomya rufifacies* throughout the study period. Other Diptera such as Muscidae, Sarcophagidae, and several species of Lepidoptera, Coleoptera and Hymenoptera were also found. Azwanzi *et al.* (2013) used rabbit carcasses in a tropical rain forest at University of Kebangsaan, Malaysia for their research and they observed similar pattern of colonization, the adult of *C. megacephala* and *C. rufifacies* were the most common necrophagous species captured during fresh and bloated stage. Trigo and Centeno (2014) worked on abnormal succession of insect fauna on pig carcasses in Argentina noted that the first insects that arrive to the carcass are flies of the families Calliphoridae and Sarcophagidae. Campobasso *et al.* (2004) noted that *Phaenicia sericata* was the first to arrive at the bloated stage and remained until the end of the active decomposition. Vanin *et al.* (2013) observed that during the winter season, the first insect activity on the burnt carcass began in the third week and it belong

to the family Calliphoridae (*Calliphora vomitoria)* but during the summer season, *Phormia regina* appeared a few minutes after the carcass exposure. Both in winter and summer, flies belonging to the first colonization wave (Calliphoridae) appeared on burnt and control pigs at the same time, whereas other species of Diptera and Coleoptera appeared earlier on burnt pigs. Abajue and Ewuim (2016) reported that the species collected over the six decomposing carcasses were *Chrysomya albiceps* (Diptera: Calliphoridae), *C. chloropyga* (Diptera; Calliphoridae), *C. regalis* (Diptera: Calliphoridae), *Isomyia dubiosa* (Diptera: Calliphoridae), *Isomyia* sp., *Sarcophaga inzi* (Diptera: Sarcophagidae), *Chrysomyza africana* (Diptera: Ulidiidae), *Musca domestica* (Diptera: Muscidae), *Hermatiaillucens* (Diptera: Stratiomyiidae), *Dermestes frischii* (Coleoptera: Dermestidae), *Necrobia rufipes* (Coleoptera: Cleridae) and *Necrobia ruficolis* (Coleoptera: Cleridae). They observed that insects found at the fresh stage were mainly few adults of blowflies (Calliphoridae) and flesh flies (Sarcophagidae); eggs of blow fly and first instar larvae of flesh flies were found during the bloating stage (day 2) while the first instar larvae of blow flies were observed during the evening of day 2. They also observed 1st instar larvae of blow flies during the late hours of day 2 through day 3 and total dispersal of the larvae to the entire body of the carrions on day 4. Adult houseflies (Muscidae), hide beetles (Dermestidae) and bone beetles (Cleridae) were observed during the bloating stages. They noted that the insects of active decay stage include, the post feeding larval and pupal stages as well as few newly emerged adult blowflies. Adult flesh flies and house flies were also spotted while hide and bone beetles were conspicuously found. Insects found at the post decay stage were few posts feeding larval, pupal and clusters of newly emerged adult blowflies on the scene and not on the carrions, during the onset of the drying stage on day 9 through day 10. Other larval and pupal stages found at this stage were the false fruit flies (Ulidiidae), soldier flies (Stratiomyiidae) and houseflies as well as the larval stages of the hide and bone beetles on the carrions during the peak of the drying stage on day 12 to day 16 through the completely dried remain. According to the work of Abajue *et al.* (2015), they observed that beetles were found to occur in succession during the decomposition stages of carrions. *Ocypus raffrayi* was the first and the only beetle collected during the fresh stage, while *Dermestes frischii* , *Necrobia ruficolis, Necrobia rufipes*, *Buphonella* sp. *Angionychus lividus*, *Zophosis* sp. and *Hister* sp. were collected during the bloating stage. *Dermestes frischii*, *Necrobia ruficolis*, *Necrobia rufipes*, *Buphonella* sp. and *Hister* sp. were also collected during the active decay stage. During the dry decay stage,

Dermestes frischii, *Buphonella* sp. *Ocypus raffrayi*, *Necrobia rufipes*, *Necrobia ruficolis*, *Angionychus lividus*, *Zophosis* sp. *Gymnopleurus* sp. *Hypocacculus* sp.and *Hister* sp. were collected. Ahmad *et al.* (2011) observed insect succession in outdoor and indoor carcass. In the outdoor fresh decomposition stage lasted between day 1-2. Within 5 minutes of death, ants of the species *Pheidologeton* sp. and *Odontoponera* sp. were attracted to the bloodstain. Within the following 30 minutes the first blowflies of the family Calliphoridae, *Hypopygiopsis violacea* (*H. violacea*) arrived and oviposition took place after 1.5 h in the oral cavity. Within hours later many calliphorines arrived and began ovipositing in the ear and the neck region. Within 6 hours after death, the eggs oviposited in the oral cavity (1st oviposition site) hatched. They observed many ants (*Pheidologeton* sp. and *Odontoponera* sp.) predating actively on the eggs. The family and species of insects collected from the outdoor condition. These insects belonged to 3 orders, namely Diptera, Coleoptera and Hymenoptera consisting of 7 families. There was also a delay of fly arrival for at least 3 days for both the replicates. Our studies indicated that the various decomposition stages in indoor condition were prolonged. Four orders of insects found indoors were: Diptera, Coleptera, Hymenoptera and Dermaptera. The order Dermaptera was found at the remains stage, while the order Diptera consisted of the family Calliphoridae, Muscidae, Sarcophagidae and Stratiomyidae. The order Coleoptera was more diverse with 6 families recovered in indoor condition: Lampyridae, Lycidae, Scarabaeidae, Silphidae, Staphylinidae and Tenebrionidae.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Study Area

The study was carried out in University of Nigeria, Nsukka, Enugu State Nigeria. Nsukka Local Government Area is located between latitude 6.5148° N to 6.5256°N and longitude 7.2388°E to 7.2568°E (Fig. 5) with land mass of 1,810km^2 and a population of 309,633 according to 2006 census (Nigeria Distribution of Regular, 2006). The elevation above sea level is 423 m (Dateandtime.info, 2017). Nsukka is characterized by green grassy vegetation consisting of grasslands, farmland, wooded shrub grassland/woodland and mature nature forests (Njokuocha, 2006). The population of Nsukka is about 70 %, consisting of small and medium scale farmers. Nsukka Local Government Area shares boundary with other Local Governments in Nsukka Senatorial Zone which include; Udenu, Igboeze-south, Isiuzo, Igboetiti and Uzouwani. There are hills and highlands in Nsukka and these result in its cooler climatic condition and erosion of different types. The two prominent climatic seasons in the area include the wet season, lasting from April to October and the dry season lasting from November to March (Ezeibe, 2010) but fluctuations have been noticed due to effects of climate change. The area is in the humid tropical region with average monthly temperature fluctuating between 24°C and 29°C and the mean annual rainfall ranging from 786 mm to 2098.2 mm (Njokuocha, 2006).

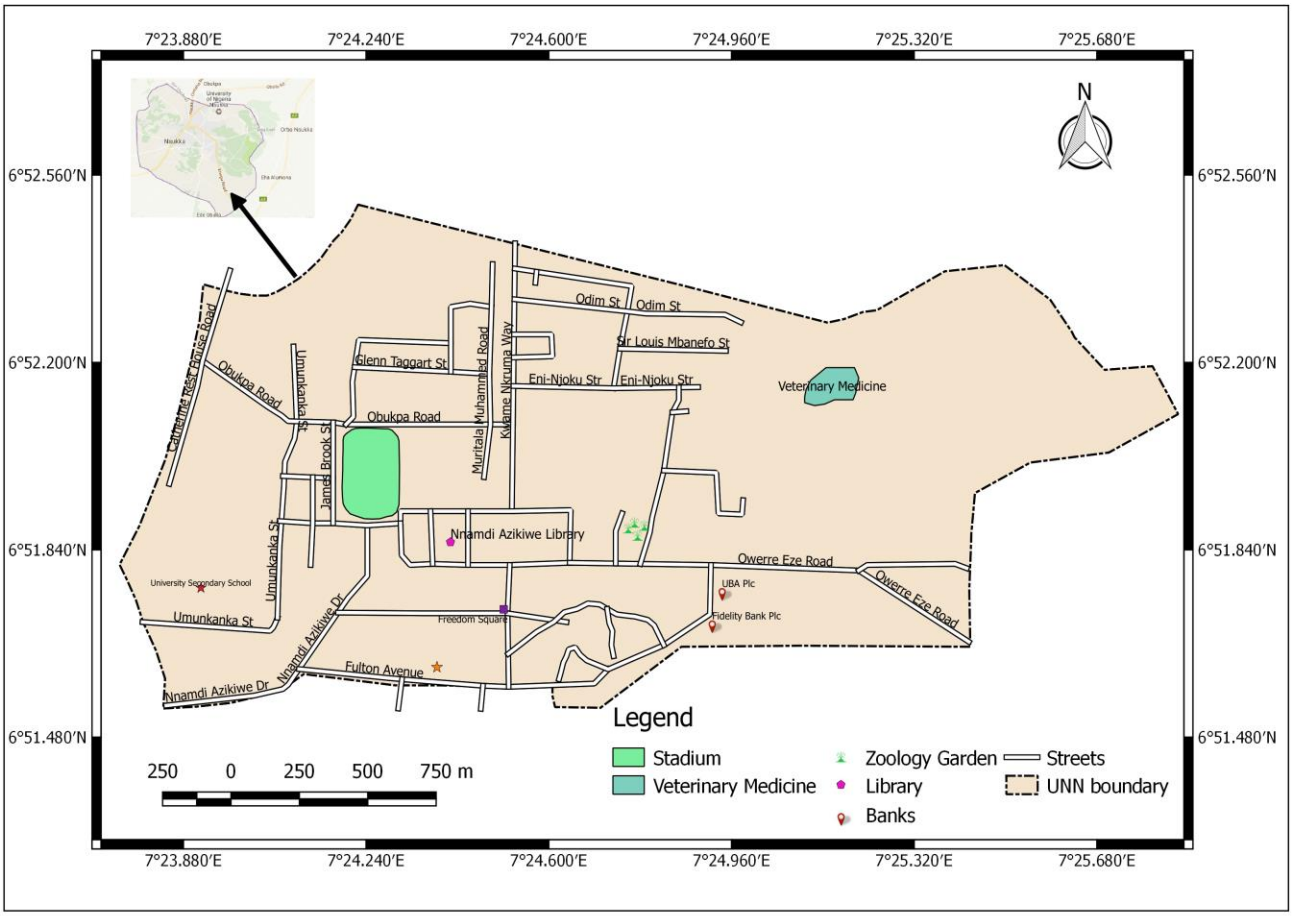


Figure 5: Map of University of Nigeria Nsukka, Enugu State showing the study sites

Source: Google Map (2017)

2.2 Experimental Animals

This research was done using nine (9) African giant rats (*Cricetomys gambianus*) as a model for human decomposition. These animals were used to study the effects of different killing methods on forensic insect fauna in a mock crime scene by observing decomposition and arthropod succession on the carrion (Ekrakene and IIoba, 2011). The animals were trapped life from the nearby bushes in Nsukka.

2.3 Killing of the Animals

The animals were killed using three methods. These include; using knife to slaughter the rat (used to simulate violent death), narcotizing with 5 ml of 2, 2-dichlorovinyl dimethyl phosphate (DDVP) common home pesticide that people use in committing suicide (used to simulate suicide death) and deprivation of oxygen (used to simulate natural death) (Ekrakene and IIoba, 2011; Sabrina *et al.,* 2015). Each killing method was replicated three times. The animals were killed at the deposition site and exposed in a built cage for decomposition. The time of death and deposition at the sites was recorded and this day was designated as day 0 (Voss *et al.,* 2008).

2.4 Setting up of the Experimental Site

Three sites A, B and C within University of Nigeria, Nsukka was used for the study. The sites were: UNN Stadium, UNN Zoological Garden and UNN Veterinary Medicine farm respectively. UNN Stadium represents site A with coordinates 6.86648 / 7.41098, UNN Zoological garden site B with coordinates 6.86627 / 7.40473 and Veterinary Medicine site C with coordinates 6.86502/7.4118. Three giant rat remains was deposited 15 m apart from one another within each site. The three giant rats were killed differently, one with a knife, second with 5ml of 2, 2-dichlorovinyl dimethyl phosphate (a common home pesticides used in suicide) and the other by oxygen deprivation. Carcass was kept in a cage of 10 cm long and 4 cm high made of wire gauze (Goff, 2009b; Ekrakene and IIoba, 2011). This was to prevent disturbances by the vertebrate scavengers. These cages were placed on the ground for easy access by the crawling insects (Jens *et al.,* 2010; Ekrakene and IIoba, 2011; Abajue and Ewuim, 2016). The distance between the sites was 50m apart.

2.5 Determination of Decomposition stages

The five stages of decomposition were determined as follows:

Fresh stage: this stage of decay started from the moment of death and ended when the bloating of the carcass was observed.

Bloating stage: this stage was observed by the swollen abdomen and arrival of different species of arthropods on the death scene. This stage was also observed by the intense unpleasant odour.

Active stage: this stage was observed by penetration of the carcass abdominal wall, resulting in the deflation of the carcass. This stage was also noted by presence of some liquid dropping from the carcass known as by-product of decomposition (BOD). It was also noted by progressive loss of carcass weight and progressive rise in internal temperature of the carcass above the ambient temperature. A drop in the ambient temperature and unpleasant odour noted the end of the decay stage.

Post decay stage: The post-decay stage was noted by gradual decline in adult and larval dipteral population. This stage is noted as when the dipteral larvae have finished feeding on the carcass, leaving behind bones, cartilage, hair, small portions of tissue

Dry skeletal remains stage: This stage was observed by the presence of bones with little cartilage remaining. BOD has also dried up during this stage and there was gradual increase in the adult and larvae of Coleoptera (Isaac *et al*., 2011).

2.6 Collection of Insects at the Mock Crime Scene

After killing and deposition of the giant rats, observations were made for the arrival of the first insect at the scene. The time of arrival of the first insect was recorded using a stopwatch and the first species to arrive was also recorded as well as the death type and the part of the body that was colonized. Therefore, daily data collections at the death scene were done as follows: temperature, relative humidity and insect evidence collections were made twice daily during the first eleven days and 2 days interval during the rest of decomposition time (Goff, 2009b; Ekrakene and IIoba, 2011). The sampling was done between 7 – 10 am and 3 – 6pm when most of the flies are active according to Slone *et al.* (2005). This is to ensure that all ranges of insects

that visited the scene were sampled as some flies may be too active to be sampled during the noon period. Sweep net was used to collect flying insects while eggs, larvae and pupae was collected using brush and forceps (Bala, and Kaur, 2015; Ries and Blochtein, 2015; Abajue and Ewuim, 2016). There were also two pitfall traps on each site to catch the nocturnal and crawling insects as well as wandering larvae (Szymon *et al.,* 2016). The samples collected were put into well labeled separate kits in the laboratory. Samples of insect fauna were collected from different natural openings of the body as well as other parts of the body. Careful observations on decomposition stages and sequence of arthropod colonization were made and pictures were taken from each site at different stages of decomposition.

2.7 Rearing of the Insects Collected to Determine PMI

Half of the larvae collected were killed in boiled water before being placed in preservative solution to avoid shrinkage (Ekrakene and IIoba, 2011). They were placed in vials with 75 % alcohol for preservation. The remaining half of the collected larvae were placed in containers and reared to the adult stage at Entomology Laboratory, University of Nigeria, Nsukka. Rearing was done according to Ekrakene and IIoba, (2011). The rearing containers were covered with muslin cloth held tightly with rubber band. The use of rubber band helped to prevent larvae and adult flies from escaping through lids and also to prevent other flies entrance into the containers (Slone *et al.*, 2005; Ekrakene and IIoba, 2011). The insects samples from different orifice (ear, nostril, eye, cut region, mouth and anus) was placed on different rearing media containing sand as the base and pieces of liver of animal purchased from the market (food source for the maggots). This was the source of their food until they emerge as adult flies (Aggarwal, 2005; Jens *et al.*, 2010; Ekrakene and IIoba, 2011). The Coleoptera larvae were place in similar container but the food source was dry fish. The containers were labeled according to the killing method and orifice from where the maggots were collected and observation was made several times to note changes in larval stages, pupation and emergence of adult insects. The containers of live maggots were monitored in the laboratory daily by measuring daily temperature of the laboratory and observable morphological changes occurring among the stages (Dhurba, 2016).

2.8 Identification of the Insects Collected

Proper identification and classification were made in the Entomology laboratory, University of Nigeria, Nsukka, using morphological keys by Bousquet (1990); Choate (2003a); choate (2003b); Terry (2006); Terry (2010); Marshall *et al.* (2011); Anthony (2011); Irish *et al.* (2014); Akbarzadeh (2015); Acikgoz, *et al.* (2016); Al-Shareef (2016). The samples were finally sent to a taxonomist at Ahmadu Bello University, Zaria for confirmation of the identified species. The total days of development was used to calculate postmortem interval (PMI). PMI was calculated by summing up the time it took from oviposition of the first insect egg till the day of the adult fly emergence. The number of each species was noted for estimation of the relative population of arthropod that visited the scene.

2.9 Statistical Analysis

Data obtained from this research was entered into Microsoft excel spread sheet and analyzed using SPSS (Statistical Package for Social Sciences), version 20. Analysis of variance (ANOVA) was used to compare the rate of decomposition on the killing methods. Duncan (1955) test was used to check the significant differences between the killing methods on the rate of decomposition. Descriptive mean was used to get the ambient temperature and relative humidity of the decomposing sites as well as that of the laboratory where larvae were reared. The analysis was done at 95 % confidence interval (level of significance, $P < 0.05$). Percentage abundance, diversity, evenness, and richness indices were calculated. Simpson's index, Gini-Simpson, Reciprocal Simpson, Shannon-Wiener diversity index, Modified Shannon-Wienner index, Berger-Parker index, McIntosh index, Margalef index, Menhinick index, Hill's family of numbers (N0, N1 and N2), Sheldons index, Heip index, Pielo's index and Simpson's index of evenness were all calculated according to the formulae listed by Ludwig and Reynolds (1988) and Krebs (2014). The formulae used include:

Simpson's index, $D = \sum_{i=1}^{s} p2$

where p is the proportional abundance of ith species

Gini-Simpson index = $1 - D$

Reciprocal Simpson or Hill's N2 = $1/D$

where D is Simpson's original index

Shannon-Wienner's index, H' = $-\sum_{i=1}^{s} pLn(p)$

where p is the proportional abundance of ith species

Modified Shannon-Wienner, H' = - $\sum_{i=1}^{s} \frac{pLn(p)}{[1-(1-p)^n}$

where p is the proportional abundance of ith species. n = total sample size

McIntosh D = $\frac{N-U}{N-\sqrt{N}}$

where U = $\sqrt{\sum n^2}$

N = total number of individuals in a population. n = numbers of individuals in the ith species

Berger – parker index of Dominance, d = $\frac{Nmax}{N}$

N_{max}= number of individual in the most abundant species. N = total number of individuals in sample

CHAPTER THREE

RESULTS

3.1 Decomposition of Rat Carcass in the Study Area

The decompositional stages observed were fresh stage, bloated, active decay, post decay and skeletonization as shown in the plats below. The mean durations of decomposition of the giant rat carcasses from fresh to skelectonization were 30.33, 48.33 and 60.00 days for violent death, natural death and suicide death respectively (Figure 6). There was significant difference in duration of overall decomposition among the three types of death ($P < 0.05$). The stage with the longest period of decomposition was skeletal stage followed by post decay stage while the least duration was observed during the fresh stage.

Fresh stage lasted between 0 – 12 hours in all treatments. During this stage, there were no physical observable changes on the carcass though putrefaction was taking place. There was no significant difference in duration of the fresh stage of decomposition ($P > 0.05$).

The bloated stage was between 1 – 2 days in violent and suicide death but extended up to 3 days in natural death. This stage is characterized by swollen stomach in the rats (looked like full blown balloon, full of gasses) and foul odour in all the rats.

The active decay stage took 3 – 9 days in violent death, 3 – 7 days in natural death but suicide death extended up to 13 days. During this stage, the foul odour was intense and the by-product of decomposition (BOD) was observed (liquid gushing out from the blown carcass). In suicide death, red liquid was coming out of the mouth and other parts of the body between 3rd and 4th day of decomposition. The insect species that perched on the liquid died immediately. During this stage, carcass of the suicide death looked red in colour, weak and broke up on touching. The duration of active decay stage of suicide death was significantly higher than other killing methods ($P < 0.05$).

The post decay stages took place between 9 – 11 days and 8 - 11 days in violent and natural deaths respectively but occurred between 14 - 21st days in suicide death. The duration of post decay in suicide death was significantly higher than other killing methods ($P < 0.05$). During this stage, the carcass looked dry, BOD has dried and foul odour had drastically reduced.

The skeletal stage of decomposition ranged from 11 – 35days (mean 30.33 days), 11 – 55 (mean 48.33 days) and 22 – 70 (mean 60 days) in violent, natural and suicide deaths respectively. The suicide death was significantly higher than the violent death ($P < 0.05$) but there was no significant difference between suicide and natural deaths ($P > 0.05$). This stage was marked by the presence of bones, cartilages and dried flesh with the increase in the number of adult and larvae of Coleoptera.

PLATES OF THE RATS IN THEIR DECOMPOSITION SITES

Fresh stage of suicide

Fresh stage of suicide death

Fresh stage of natural death

Early bloating stage of suicide death

Bloating stage of violent death

Late bloating stage of suicide death with mass of dead flies

Bloating stage of natural death

Decay stage of violent death

death

Post decay of violent death

Post decay of natural death

Skeletal stage of violent death

Post decay of suicide death

Skeletal stage of natural death

Skeletal stage of s

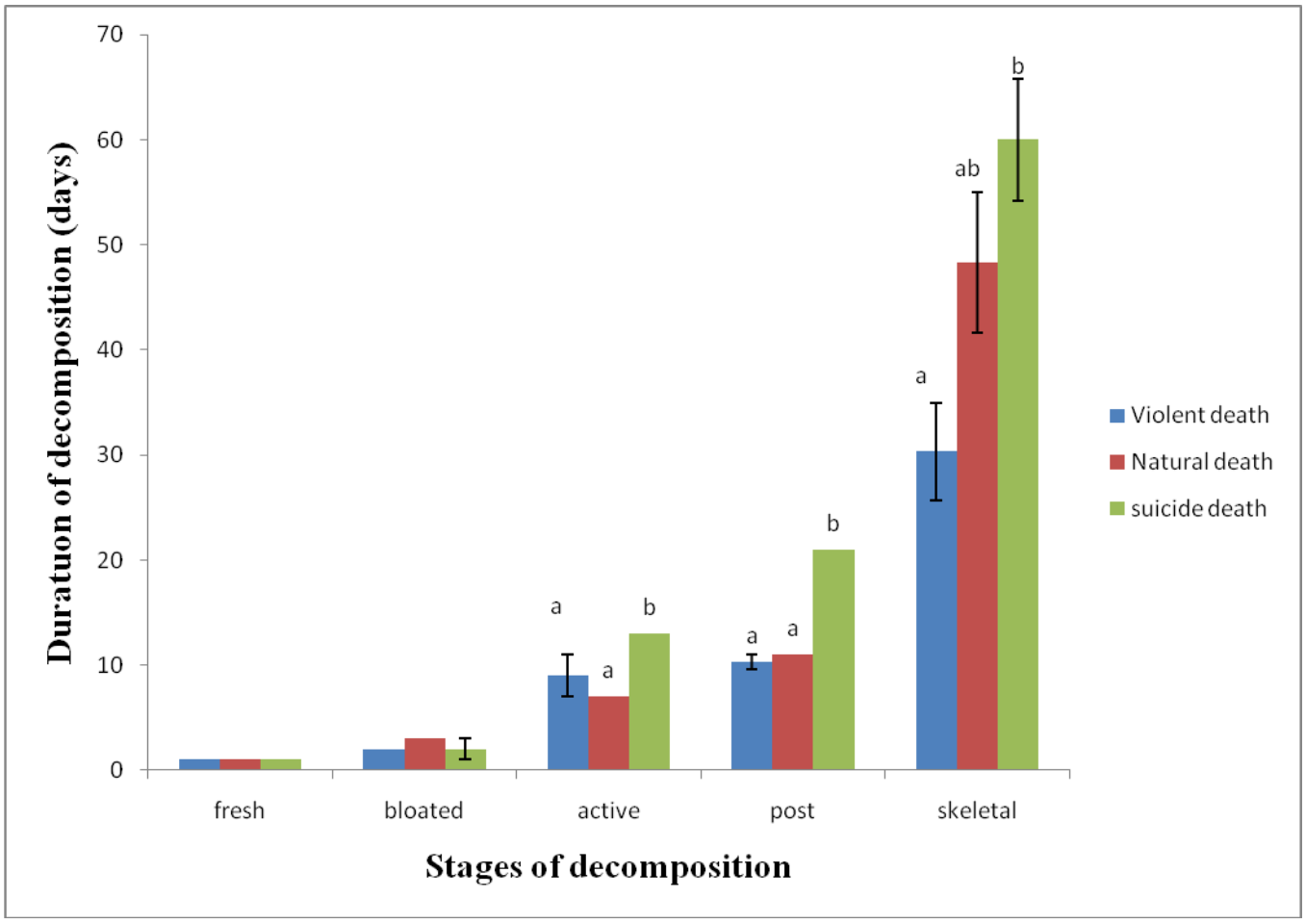


Figure 6: Mean duration of decomposition of the giant rat carcasses. Bars with different labels were significant different ($p < 0.05$)

3.2 Insect Fauna of the Decomposition Rat Carcass

The methods of killing employed influenced the arthropod population on the decomposition remains. A total of 5064 arthropods were collected during the period of study. These comprised the necrophagous arthropods, predators and parasites, and adventives or accidental arthropods. Some of the necrophagous species include; *Chrysomyia chloropyga*, *C. albiceps*, *Lucillia sericata*, *L. cuprina* (Calliphoridae), *Oestrus ovis* (Oestridae), *Sarcophaga inzi*, *S. exuberans* (Sarcophagidae), *Musca domestica*, *Morellia nilotica* (Muscidae), *Dermestes maculates*, *D. frichii*, *D. atar* (Dermestidae) and *Necrobia rufipes* (Cleridae). The predators and parasites of necrophagous species include: *Sarcophaga inzi* and *S. exuberans.* The family Dermestidae, Histeridae and Scarabaeidae adults as well as the Formicidae were also predatory on necrophagous species. The adventive and accidental species were *Lethocerus* sp., *Laemophaeus fasciatus*, *Gryllus* sp., *Scolopendra gigantean* (centipide), *Anadenobolus* sp. (milipide), *Araneus* sp. (spider), *Periplaneta* sp. and *Lymantria dispar* (moth). All the arthropod species collected on the decomposing giant rat remains are shown in Table 1.

The most dominant insect species from the three killing methods was *Chrysomyia albiceps* while the least dominant species were *Laemophoeus fasciatus*, *Sandalus niger*, *Harpalus honestus*, *Prosoestus* sp., *Lema dentipes*. The number of *Chrysomyia albiceps* was higher in violent death while the least were found in suicide death. Most of the adventitive or accidental arthropods visited the suicide death and natural death such as *Laemophoeus fasciatus*, *Sandalus niger*, *Harpalus honestus*, *Prosoestus* sp., *Lema dentipes*, *Scolopendra gigantean* (centipide), *Anadenobolus* sp. (milipide), *Araneus* sp. (spider), *Periplaneta* sp. and *Lymantria dispar* (moth). Only small number of adventitive or accidental species visited the violent death such as *Gryllus* sp., *Periplenata* sp. and *Araneus* sp. (spider).

Emerged adults collected from the reared larvae are represented in Fig. 6. There were many arthropod species that fed on the carcass but only few of them bred on the carcass. In the present study, only the ones that breed on the carcass were used in determination of postmortem interval. The insect fauna of the violent death was significantly difference from other killing methods ($P < 0.05$). The significant different was due to the family Calliphoridae which were the highest colonizers. The Calliphoridae family formed maggot masses in violent death. There were many maggots on the natural death but it could not form maggot mass. The suicide death had the least

number of Calliphoridae and other insect families. Many Calliphoridae and other families were attracted to the suicide death but they died on the spot thus affecting the type and population of species that breed on the suicide death. The following dead insects were collected on the suicide death between the first and second day of decomposition; Calliphoridae (773), Muscidae (156), Sarcophagidae (74), Coleoptera (5), Formicidae (*Camponotus sericeus*) (402) and Formicidae (*Oecophylla* sp) (1).

The family Sarcophagidae was also higher in violent death and the least was observed in suicide death though there was no significant difference among the killing methods ($P > 0.05$). Natural death had the highest number of Dermestidae and Cleridae while the least was observed in suicide death but there was no significant difference ($P > 0.05$) among the killing methods.

Table 1. Arthropod Fauna of the Giant Rat Carcass during the Study Period

Order	Family	Species	Total number of species
Diptera	Calliphoridae	*Chrysomyia chloropyga* (Wied.)	460
		Chrysomyia albiceps (Wied.)	1189
		*Stomorhina rugosa (*Bigot.)	25
		Bengalia peuhi (Villen.)	21
		Lucillia sericata (Meig.)	122
		Lucillia cuprina (Wied.)	5
		Pollenia sp. (Fab.)	7
	Phoridae	*Megaselia scalaris* (Loew.)	28
Diptera	Sarcophagidae	*Sarcophaga exuberans* (Pand.)	97
		Sarcophaga inzi (Curron)	448
	Muscidae	*Musca domestica* (Linn.)	325
		Morellia nilotica (Walk.)	161
	Oestridae	*Oesterus ovis (*Linn.)	3
Coleoptera	Dermestidae	*Dermestes frischii (*Klug.)	195
		Dermestes maculatus (DeG.)	99
		Dermestes ater (DeG.)	18
		Trogoderma granaries (Everts.)	16
	Cleridae	*Necropis rufipies* (DeG.)	43
	Curculimidae	*Prosoestus* sp. (Faust.)	1
	Chrysomelidae	*Lema dentipes* (Jac.)	1
	Scarabaeidae	*Gymnopleurus fulgidus* (Oliv.)	5
		Gymnopleurus capensis (Ferr.)	11
		Gymnopleurus laevicollis (Cast.)	39
		Phanaeus igneus (Mac.)	2
		Ateuchetus laticollis (Fab.)	3
		Euphoria kernii (Hald.)	5
	Trogoidae	*Omorgus bachorum* (Eric.)	8

	Histeridae	*Platysoma leconti* (Mars.)	16
		Saprinus sp. (Erichson)	9
		Euspilotus assimilies (Pay.)	8
		Euspilotus scrupularis (Fisher)	3
		Margarinotus brunneus (Fab.)	2
		Harpalus honestus (Dufisch.)	1
	Carabidae	*Galeritiola africana* (Dejean)	2
	Cucujidae	*Laemophoeus fasciatus* (Melsh.)	1
	Rhipiceridae	*Sandalus niger* (Knoch)	1
Hymenoptera	Formicidae	*Oecophylla longinoda* (Fab.)	378
		Camponotus maculatus (Erich.)	155
		Camponotussericus (Fab.)	866
		Monomorium minimum (Buckley)	205
	Vespidae	*Vespa* sp. (Linn.)	2
Orthoptera	Gryllidae (Cricket)	*Gryllus bimaculatus* (Linn.)	31
Lepidoptera	Erebidae (Moth)	*Lymantria dispar* (Linn.)	3
Hemiptera	Belostomatidae	*Lethocerus sp.* (Mayr)	4
Dictyoptera	Blattidae	*Periplaneta* sp. (Fab.)	11
Arachnidae	Araneae (spider)	*Araneus* sp. (Clerck)	23
Scolopendromorpha	Scolopendridae (Centipede)	*Scolopendra gigantea* (Linn.)	3
	Rhinocricidae (Milipede)	*Anadenobolus* sp. (Von Porat)	3
Total			5064

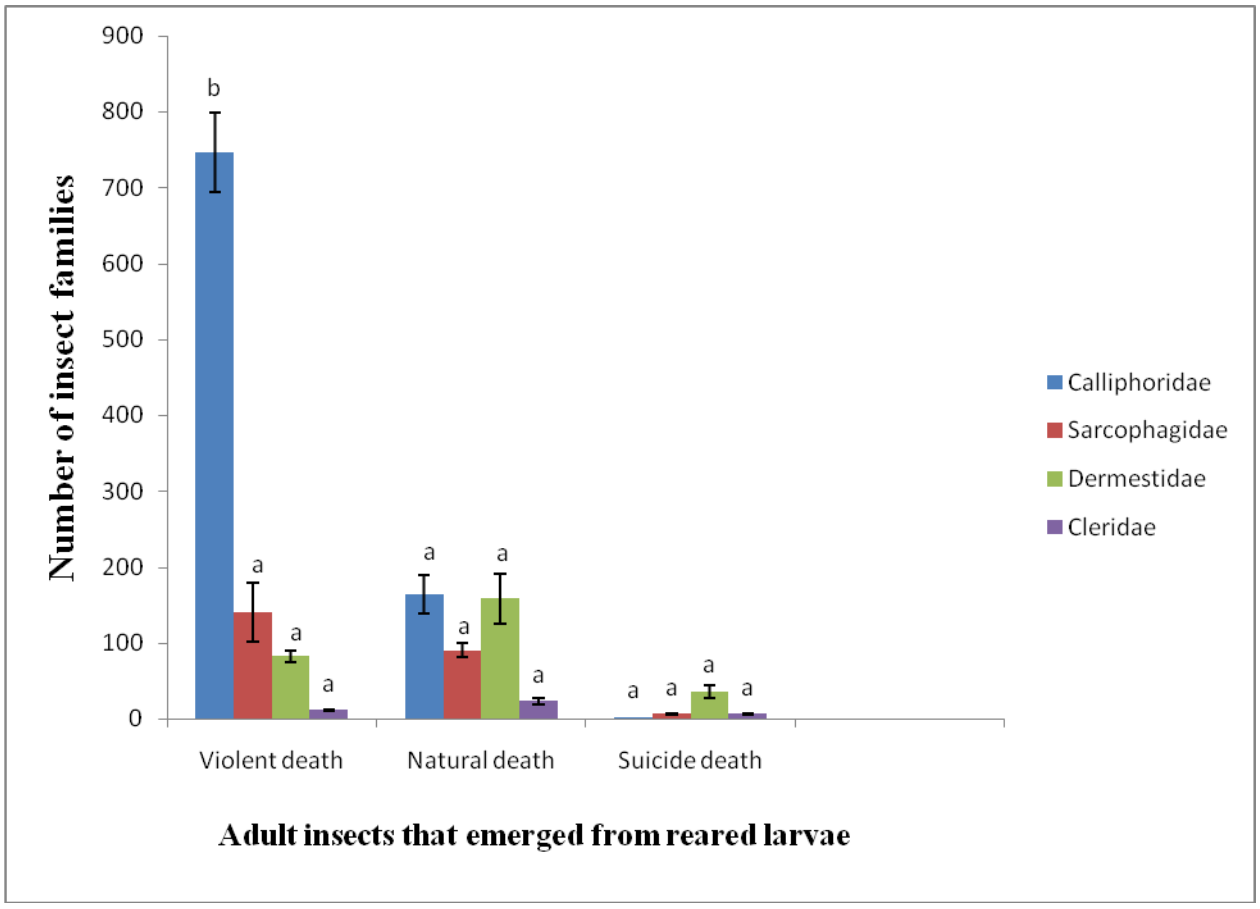
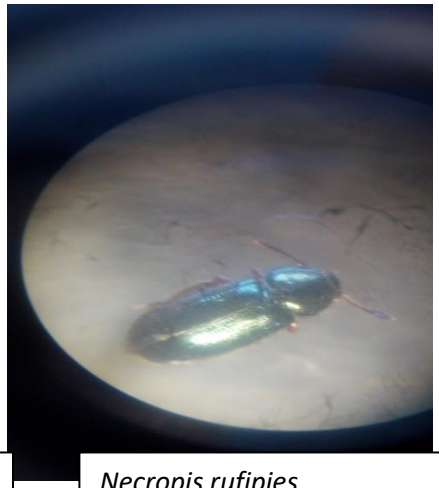


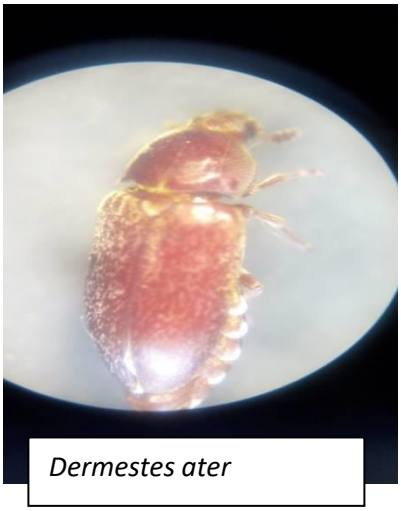
Figure 7: Insect families that emerged from the reared insect larvae. Bars with different labels were significantly different ($p < 0.05$)

SPECIES OF INSECTS COLLECTED FROM THE DECOMPOSING CARCASS

Dermestes frischii

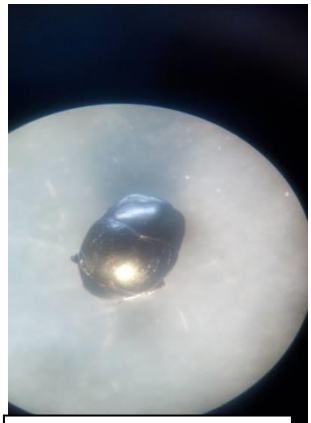


Necropis rufipies

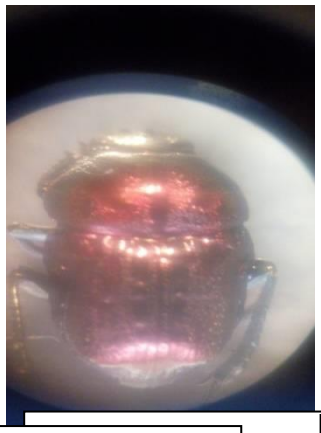


Dermestes ater

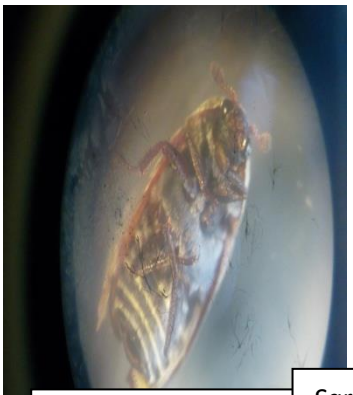
Harpalus honestus



Pollenia sp.

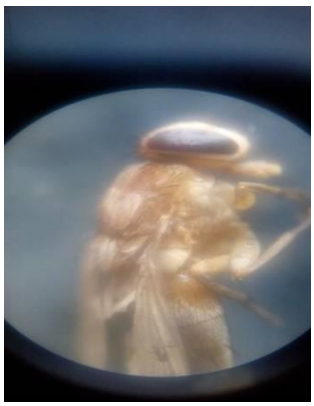


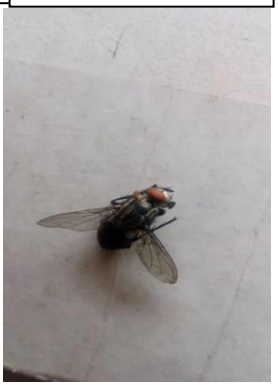
Calliphora sp.



Calliphora sp.

Sarcophagi inzi





3.3 Insect Succession on the Decomposing Giant Rat Carcass

There was succession of insects that colonized the decomposing carcasses in different killing methods. As soon as death occurred, the carcasses were observed for the first insect colonizers and their successional pattern at the mock scene.

In violent death *Monomorium minimum* (Hymenoptera: Formicidae) were the first arthropod to visit the rat killed with knife (violent death) after 5 minutes. They were seen licking the blood on the carcass. The blow fly (Diptera: Caliphoridae) arrived at the scene after 29 minutes and perched on the open wound.

In natural death, *M. minimum* (Hymenoptera: Formicidae) visited the carcass after 7 minutes. It was crawling and licking the sweat on the carcass. *Camponotus perrisi* also visited the carcass dring this stage. The house fly (Diptera: Muscidae) visited the carcass after 20 minutes. It only perched on the carcass and left after few seconds.

In suicide death, the *Camponotus sericeus* (Hymenoptera: Formicidae) visited the carcass after 2 hours 45 minutes but died instantly on the scene. The flesh fly (Diptera: Sarcophagidae) was seen after 24 hours though it still died on the spot.

The blow flies that colonized the carcass laid eggs on the natural orifice of the body within 24 hours of death. In violent and natural death, dipteral eggs were found on the mouth, head, open wound (only on the violent death), anus (only on the natural death) and on other body parts. The ants were found eating the flesh on the ears. Neither eggs nor larvae were found on the suicide death during the fresh and bloating stages of decomposition. The Sarcophagidae also visited the carcass during this early stage of decomposition but did not lay eggs immediately. Histeridae adults also visited the carcass on the 2nd day of decomposition (bloating stage).

During the active decay stage, the greatest numbers of dipteral larvae were observed except in suicide death. Maggot masses occurred in the violent death carcass and many larvae in the natural death carcasses, though they could not form maggot mass.

Although in suicide death, eggs and larvae were collected during the late active decay stage, they were never found on the mouth, anus or on the head rather eggs and larvae were found on other parts of the body apart from the orifice of the body. The head was not colonized by any insect species until the post decay stages. In suicide death, the insect visitors stopped dying on the 4th

day but could not lay eggs until the 6th day. All the larvae collected on the 6th day were dead, the viable eggs and larvae were collected on the 7th day of decomposition. The Sarcophagidae and Oestridae laid eggs during the active decay stage of decomposition. In natural and violent death, eggs and larvae of Sarcophagidae were collected on the evening of the 3rd day but in suicide death, it was collected on the 11th day of decomposition. The Muscidae larvae were never collected over the nine carcasses though the adults were present from the fresh stage to the end of carcass decomposition. This showed that house fly only fed on the carcass but did not breed on it. Other arthropods that visited the carcass during this stage were Histeridae, Dermestidae, Cleridae, Scarabaeidae, Belostomatidae, Trogidae and Rhipiceridae respectively. The family Dermestidae, Cleridae and Histeridae were found in all the killing methods. The family Trogidae and Scarabaeidae were found in the natural and suicide death while Belostomatidae and Rhipiceridae were only found in the natural death. Dermestidae visited the carcass on the 5th day and stayed till the skeletonization stage though their eggs and larvae were only found during post decay stage.

Post decay stage was marked by the gradual decrease in dipteral larvae or pupa and presence of coleopteral adults and larvae. The coleopteran adults fed on the bones, fur and other dry flesh that was left by the dipteral larvae. Adult Dermestidae and Cleridae as well as few eggs were found during this stage.

During the skeletal stage of decomposition, the greatest number of coleopteran larvae was found on the natural death and the least larvae were observed on the suicide death though it took the longest period of decomposition. The successional pattern of the necrophagous species during different stages of decomposition is shown in Table 2.

Table 2. Stages of insect (Larvae, Pupae and Adult) species present during decomposition in their successional pattern

Species	Fresh			Bloated			Active			Postdecay			Skeletal		
	L	P	A	L	P	A	L	P	A	L	P	A	L	P	A
Lucillia sericata	□	□	⊠	▥	□	⊠	▥	▩	⊠	□	▩	⊠	□	□	⊠
Chrysomyia albiceps	□	□	⊠	▥	□	⊠	▥	▩	⊠	□	▩	⊠	□	□	⊠
Chrysomyia chloropyga	□	□	⊠	▥	□	⊠	▥	▩	⊠	□	▩	⊠	□	□	⊠
Chrysomyia putoria	□	□	⊠	▥	□	⊠	▥	▩	⊠	□	▩	⊠	□	□	⊠
Phumosia imitans	□	□	⊠	▥	□	⊠	▥	▩	⊠	□	▩	⊠	□	□	□
Pollenia sp.	□	□	⊠	▥	□	⊠	▥	▩	⊠	□	▩	⊠	□	□	□
Stomorhina rugosa	□	□	⊠	▥	□	⊠	▥	▩	⊠	□	▩	⊠	□	□	□
Oestrus ovis	□	□	⊠	▥	□	⊠	▥	▩	⊠	□	▩	⊠	□	□	□
Sarcophaga exuberans	□	□	⊠	□	□	⊠	▥	□	⊠	□	▩	⊠	□	□	⊠
Sarcophaga inzi	□	□	⊠	□	□	⊠	▥	□	⊠	□	▩	⊠	□	□	⊠
Musca domestica	□	□	⊠	□	□	⊠	□	□	⊠	□	□	⊠	□	□	⊠
Morellia nilotica	□	□	⊠	□	□	⊠	□	□	⊠	□	□	□	□	□	□
Dermestes frichiis	□	□	□	□	□	□	□	□	⊠	▥	▩	⊠	▥	▩	⊠
Dermestes atar	□	□	□	□	□	□	□	□	⊠	▥	▩	⊠	▥	▩	⊠
Dermestes maculatus	□	□	□	□	□	□	□	□	⊠	▥	▩	⊠	▥	▩	⊠
Trogodema granarius	□	□	□	□	□	□	□	□	⊠	▥	▩	⊠	▥	▩	⊠
Necrobis rufipies	□	□	□	□	□	□	□	□	⊠	▥	▩	⊠	▥	▩	⊠

Legend: □ Neither larvae (L), Pupa (P) nor Adult (A) were present. ▥ Larva present, ▩ Pupa present, ⊠ Adult present.

3.4 Arthropod Species Diversity during Decomposition of the Carcass

The diversity of total arthropod species collected from the carcasses is shown in table 3. There was significant variation on the species composition and diversity during the decomposition stages of the three killing methods of rats. The natural death had the highest species diversity/distribution using the following indices; Simpson's (0.086336), Hill's (15.45187), Shannon-Wiener (2.737732), Berger-Parker (0.166166), Mclntosh (0.729243) while the violent death had the least species diversity; Simpson's (0.189724), Gini-Simpson's (0.810276), Berger-Parker (0.391773) and Mclntosh (0.579475). The natural death carcass had the highest number of species when compared to other types of death, Margalef (5.067503) and Menhinick (1.138990), followed by suicide death Margalef (4.32776) and the least being violent death Margalef (3.560755). The species were more evenly distributed on the natural death carcass; Equitability of Pielo (0.763978), Simpson's Evenness (0.321742) and Sheldon's (0.429219). The suicide death had the least species evenness among the killing methods; Heip's (0.412911), Equitability of Pielo (0.615562) and Sheldon's (0.254919). Although the sample size of the natural death was the smallest of other killing methods, it had the highest species diversity, richness and the most evenly distributed species.

The adult insects that emerged from the reared larvae collected from the decomposing carcass are shown in table 4. The data included only the adult arthropod species that emerged from the reared larvae. There were more numbers of species in violent death than in other killing methods, Margalef (1.898363) and Menhinick (0.456145) while suicide death had the least species richness, Menhinick (1.083473). The natural death had the highest species diversity, Simpson's (0.1484), Gini-Simpon's (0.8516), Mclntosh (0.644838), Hill's 1^{st} Order No (8.027461) and Shannon-Wiener (2.08287).

Table 3. Species diversity during decomposition of the giant Rats carcass

Indices	**Types of death**

Richness	**Violent death**	**Natural death**	**Suicide death**
Margalef	3.560755	5.067503	4.327726
Menhinick	0.701121	1.138990	0.688795
Diversity indices			
Simpson's (D)	0.189724	0.086336	0.159873
Gini-Simpson's (1 - D)	0.810276	0.913664	0.840127
ReciprocalSimpson (1/D)	5.270826	11.58271	6.25496
Hill's 1st Order No. (N1)	9.574319	15.45187	8.922166
Shannon-Wiener (H')	2.259086	2.737732	2.18854
Modified Shannon	2.259086	2.737732	2.18854
Berger-Parker	0.391773	0.166166	0.265298
Mclntosh	0.579475	0.729243	0.612207
Evenness			
Simpson's Evenness	0.195216	0.321742	0.178713
Equitability of Pielo	0.685436	0.763978	0.615562
Sheldon's	0.354604	0.429219	0.254919
Heip's	0.329782	0.412911	0.233005
Hill's	0.550517	0.749599	0.701058
Modified Hill's	0.498095	0.732272	0.663324
Sample size	1483	999	2582
Species No.	27	36	35

Table 4. Species diversity of arthropods that emerged from the reared larvae collected from the decomposing carcass

Indices		**Type of death**	
Richness	**Violent death**	**Natural death**	**Suicide death**
Margalef	1.898363	1.810596	1.889419
Menhinick	0.456145	0.575356	1.083473
Diversity			
Simpson's (D)	0.333847	0.1484	0.277463
Gini-Simpon's (1 - D)	0.666153	0.8516	0.722537
Reciprocal Simpson (1/D))	2.995382	6.738542	3.604088
Hill's 1^{st} Order No. (N1)	4.885327	8.027461	4.863767
Shannon-Wiener (H')	1.586237	2.08287	1.581814
Modified Shannon	1.586237	2.08287	2.491821
Berger-Parker2	0.543524	0.209195	0.376812
Mclntosh	0.435797	0.644838	0.529748
Evenness			
Simpson's Evenness	0.213956	0.561545	0.400454
Equitability of Pielo	0.601062	0.838208	0.719914
Sheldon's	0.348952	0.668955	0.540419
Heip's	0.298871	0.63886	0.482971
Hill's	0.613139	0.839436	0.741008
Modified Hill's	0.5135569	0.816588	0.673976
Sample size	942	435	69
Sample No.	14	12	9

3.4. Postmortem Interval

Table 5. Postmortem Interval of the Giant Rats

Killing methods	Date of death	Date of collection	Date of pupation	Date of emergence	Postmortem interval (PMI)	Species
Violent death	11.00am 6/3/2017	7/3/2017 (Eggs and larvae)	12/3/2017	4.00pm 14/3/2017	9 days	*Chrysomyia albiceps*
Natural death	2.00pm 6/3/2017	7/3/2017 (Eggs)	12/3/2017	7.20am 15/3/2017	9 days	*Chrysomyia chloropyga*
Suicide death	9.00am 14/3/2017	20/3/2017(Eggs and larvae)	2/4/2017	7.00am 8/4/2017	25 days	*Lucilia sericata*

3.5 Temperature and Relative Humidity of the Sites during the study period

There was similar temperature record during the decomposition of the carcass (Table 6). The mean temperature of surrounding environment of the study were 31.94°C, 30.42°C and 30.80°C for site A, site B and site C carcass respectively. The minimum and maximum temperature observed was 25°C and 39°C respectively. The Laboratory where the larvae were reared had mean temperature of 26.45°C with minimum and maximum temperature of 23°C and 32°C respectively. The mean relative humidity of the decomposing carcass for site A, site B and site C carcass were 70.35%, 75.00% and 69.7% respectively (Table 7). The minimum and maximum relative humidity was 56 and 80 respectively. The mean relative humidity of the rearing Laboratory was 74.65% with minimum and maximum of 69% and 82%. The temperature and relative humidity of the Laboratory ensured that there was no dormancy (delay) in development of the larvae.

Table 6. Environmental temperature readings during decomposition of the carcass and rearing of larvae

Killing methods	Minimum (°C)	Maximum (°C)	Mean (°C)
Site C	25	37	30.80
Site B	24	35	30.42
Site A	24	39	31.94
Laboratory	23	32	26.45

Table 7. Relative humidity of the decomposition carcass and the Laboratory where larvae were reared

Killing methods	Minimum (%)	Maximum (%)	Mean (%)
Site C	57	80	67.70
Site B	69	80	75.00
Site A	56	80	70.35
Laboratory	69	82	74.65

CHAPTER FOUR:

DISCUSSION

In this study, the decomposition of the carcass was observed in five (5) progressive and successive stages: fresh, bloating, active decay, post decay and skeletal stage which followed the description by Nazni *et al.* (2011); Zahid *et al.* (2013); Robin and Nor (2015) and Mohammed *et al*. (2015). These stages were marked by the presence/ absence of odour, morphological observation of decomposing carcass; insect type and developmental stages present in relation to the activities of the insects.

There was significant variation in the decomposition duration according to the killing methods. The violent death had the shortest mean duration (30.33 ± 4.67 days), followed by the natural death (48.33 ± 6.67 days). The suicide death had the longest mean decomposition period (60.00 ± 5.77 days). This is similar to the observation of Ekrakene and IIoba (2011) who observed 32 – 45 days on slaughtered carcass and 65 – 70 days on carcass killed with monocrotophos poisoning (venous poisoning). The shortest period that was observed in violent death could be attributed to the presence of open wound. The open wound was the site for maggot masses of Calliphoridae which voraciously ate up about 80% of the carcass during the active stage of decomposition. The post decay and skeletal stages constituted only the bones and cartilages which were fed on by the Coleoptera; hence taking the shortest period of decomposition. The longest period noted in suicide death could be attributed to the chemical (2, 2-dichlorovinyl dimethyl phosphate) which hindered insect activities; hence lowered decomposition rate. In suicide death, the viable larvae of Calliphoridae were not found on the carcass until the 7^{th} day of decomposition and no maggot mass was formed.

The fresh stage of decomposition took 0-12 hours which is similar to the observation of Ekanem and Dike (2010) and Abajue and Ewuim (2016). Bloated stage occurred between 1 – 2 days in suicide and violent death but extended to the 3^{rd} day in natural death, which is similar to the observation of Sukchit *et al.* (2015). Active decay stage took place between 3 – 9 days in violent death, 3 – 7 days in natural death and 3 – 13 days in suicide death. The delay in the suicide death decomposition could be attributed to the chemical used in killing the rat which prevented the blow fly colonizers from laying eggs. This resulted in few viable larvae, which fed on the carcass thus increasing the duration of decomposition in suicide death. The post decay stages took place between 9 – 11 days and 8 - 11 days in violent and natural death but extended to 14 – 21 days in

suicide death. The skeletal stage of decomposition lasted between 11- 35days (mean 30.33 ± 4.67 days), 11 – 55 days (mean 48.33 ± 6.67 days) and 22 – 70 days (mean 60.00 ± 5.77 days) in violent, natural and suicide death respectively. The longest period of decomposition in suicide death could be as a result of the chemical used in killing the rat and the dosage used, which deter Coleoptera adults from laying eggs. This made little Coleoptera larvae to feed on the carcass thus increasing the duration of decomposition. (The suicide death carcass in site C had neither Diptera nor Coleoptera colonizers. Insects visitors only fed on the carcass but could not breed on it).

In this study, the total number of arthropod species collected was 48 species, constituting of 23 families and 10 orders (Table. 1). The natural, violent and suicide death had 36, 27 and 35 number of species respectively (Table. 3). The total number of individual arthropods (sample size) collected during the study was 5064 with 1483, 999 and 2582 for violent, natural and suicide death respectively.

The dominating insect orders were: Hymenoptera, Diptera and Coleoptera, which occurred in their order of successional pattern. This result is in line with earlier investigators including: Payne (1965), Aggarwal (2005), Okiwelu (2008), Ekrakene and IIoba (2011), Okiwelu (2013) and Sukchit *et al.* (2015). Diptera were 4 families comprising Calliphoridae, Sarcophagidae, Muscidae and Oestridae. The Coleoptera collected in this study comprised 10 families and 23 species. The families include Dermestidae, Cleridae, Scarabaeidae, Carabeidae, Trogidae, Curculimidae, Chrysomelidae, Histeridae, Curcujiformia and Rhipiceridae.

The family of Hymenoptera was represented by Formicidae and Vespidae. The family Formicidae was represented by *Monomorium minimum, Camponotus sericeus, Oecophylla* sp, and *Camponotus consobrinus.* The earliest ant visitors were *Monomorium minimum* and *Camponotus sericeus* which visited the natural and violent death. The *M. minimum* (ants) visited the carrion after 5 minutes of death as also observed by Ekrakene and IIoba (2011). In suicide death, *Camponotus sericeus* (giant black ant) visited the scene after 2 hrs 46 minutes and this ant (*Camponotus sericeus*) died after few minutes showing that the presence of chemicals on the decomposing carcass poisoned the insect and hindered its metabolic activities. The total number of ants collected during this study were; 866, 205, 378 and 127 for *Camponotus sericeus, M. minimum, Oecophylla* sp. and *C. consobrinus* respectively. In violent death, *M. minimum and Camponotus sericeus* were found lapping blood from the open wound and those that gushed on

the ground. The *Camponotus sericeus* was also observed feeding on the dipteral larvae and eating off the fur on the carrion. The *Oecophylla* sp. was also among the earliest visitors but occurred only in one site (site B of suicide death). This showed that their presence is not carrion oriented; rather occur by presence of other ecological factors which attract them. They were collected during the fresh stage and were presence throughout the stages of decomposition. They occurred in mass and also fed on dipteral larvae. The predatory nature of Formicidae was also observed by Ekanem and Dike (2010), Ekrakene and IIoba (2011), Okiwelu (2013) and Ahmed *et al.* (2011). The family Vespidae was grouped as accidental species because they were not observed playing any role in decomposition.

Blow flies visited the carcass during fresh stage; green bottle fly visited the violent death within 29 minutes of death and after 24 hrs in suicide death. The natural death was visited by house fly after 20 minutes of death. The blowfly larvae were collected in less than 24 hrs in violent death. In suicide death, larvae were collected from the carcass on the 6^{th} day of decomposition (but all larvae collected were dead). The viable larvae were collected on the 7^{th} day of decomposition. The family of Calliphoridae remains the most important of other families of Diptera in forensic sciences as it was the first carrion colonizer. In the present study, the species of Calliphoridae observed were *Lucilia sericata, Lucilia cuprina, Chrysomyia chloropyga, Chrysomyia albiceps, Stomorhina rugosa, Megaselia* sp., *Bengalia peuhi and Pollenia* sp. The first colonizers among these Calliphoridae were the genus *Lucilia* and *Chrysomyia*. They occurred in all the types of death and in all the working sites. They came minutes after death and remained present except in skeletal stages. They were the main necrophagous insects, the larvae actively fed on the carrion; causing about 80% of decomposition. In violent death, the larvae formed maggot masses in the open wounds, which increased the internal temperature of the carrion from ambient temperature of 26°C – 31 °C; thus increased decomposition rates. This was observed in violent death which had only 30.33± 4.67 days mean period of decomposition. This result is similar to Ekrakene and IIoba (2011) who observed short period of decomposition of 32 – 45 days in slaughtered carcass. *Chrysomyia albiceps* was the most abundant and most diverse species in the whole killing methods. This observation is in agreement with the work of AL-Mesbah (2010), Rafael and Liliana (2013), Abajue and Ewuim (2016), Abimel *et al.* (2016) and Albushabaa (2016). Earlier researchers in Nigeria also got *Chrysomyia chloropyga* and *Chrysomyia albiceps*, including

Okiwelu (2008); Ekanem and Dike (2010); Abajue and Ewuim (2016); Ado-bala *et al.* (2016) and Feugang *et al.* (2012).

The Sarcophagidae families collected include *Sarcophaga inzi* and *Sarcophaga exuberans*, which is in line with the observation of Ado-bala *et al.* (2016) and Abajue and Ewuim (2016) who collected *S. exuberans* and *S inzi* respectively. The Sarcophagidae also visited the scene during the fresh stage but laid eggs on the 3rd day of decomposition (active stage) which is similar to the work of Ekrakene and IIoba (2011) that observed Sarcophagidae larvae between 2 – 4 days of decomposition. However, from this observation, it can be deduce that Sarcophagidae may not be suitable for the determination of PMI since they are not the first insect visitors and their larvae appear during active decay when the blow fly larvae are still present. The Sarcophagidae larvae were predatory on the Calliphoridae larvae. The presence of Sarcophagidae larvae on the 3rd day of decomposition led to the progressive decrease in the Calliphoridae larvae on the 4th day. The family Oestridae belongs to *Oesterus ovis*. This species also bred on the carcass during the active decay stage. Muscidae family was represented by *Musca domestica* and *Morelia nilotica* which were collected during the fresh and bloating stages of decomposition. This study reveals that though Muscidae fed on carcass during decomposition, they did not bred on the carcass, thus may not be of forensic importance in the determination of PMI using maggot age and development. This observation concurred with the work of Aggrawal (2005), AL-Mesbah (2010) and Ado-bala *et al.* (2016) but in contrast to the observation of Ekrakene and IIoba (2011).

However, among the 10 families of Coleoptera found in this study, the dominant family was Dermestidae having 328 individuals. The family Dermestidae includes the following species: *Dermestes frischii*, *D. maculatus*, *D. atar* and *Trogoderma granaries*. The adults were first collected on the 5th day of decomposition and throughout post decay and skeletonization period. This is in agreement with Ekrakene and IIoba (2011) who observed Dermestidae between 5–11 days of decomposition. Dermestidae are the hide beetle which feed on carrion and animal products which was in agreement with this present research as Dermestidae were the principal Coleoptera that bred on the entire carcass despite the killing methods. *Dermestes frischii* and *Dermestes maculatus* were the first emerged species and dominant of all the coleopterans collected in this study (total no. of individual was 195 and 99 respectively). This result reveals that the two species can be used to predict the estimated time period of death using insect

successional wave. Earlier researchers including Ekrakene and IIoba (2011), Okiwelu (2013), Rosina *et al.* (2013), Vanin *et al.* (2013), Zahid *et al.* (2013), Bala and Kaur (2015), Ries and Blochtein (2015), Abajue and Ewuim (2016) and Defilippo *et al.* (2016) obtained *D. frichii* and *D. maculatus* in their study. Cleridae that was collected in this study is *Necrobis rufipes* (ham beetle). This species bred on the carcass of all the killing methods as observed by Michaud, *et al* (2010); Ekrakene and IIoba (2011); Rafael and Liliana (2013); Zahid *et al.* (2013), Jadav and Sathe (2015) and Abajue and Ewuim (2016). *Necrobis rufipes* (Cleridae) individual were 43 in number, being second in abundance of all Coleoptera. They were among the necrophagous insects that bred on the carcass in all the killing methods during the post decay and skeletal stage of decomposition. The family Histeridae was observed during the bloating stage, constituting 6 species namely: *Platysoma leconti, Saprinus impressus, Euspilotus assimilies, Euspilotus scrupularis, Margarinotus brunneus and Harpalus honestus.* This is similar to the work of Ekanem and Dike (2010), Michaud *et al.,* 2010; Aballay *et al.* (2013), Lavinia and Corneliu (2013), Jadav and Sathe (2015), Albushabaa (2016), Szymon *et al.* (2016) and Mashaly (2017). The species visited the carcass but did not breed on the carcass. The family Scarabaeidae was represented by seven (7) species including: *Gymnopleurus fulgidus, Gymnopleurus capensis, Gymnopleurus laevicollis, Phanaeus igneus, Ateuchetus laticolis,* and *Euphoria kernii.* Their larvae were not collected over the carrion; therefore, they only used the carcass for food and shelter as noted by Zahi *et al.* (2013), Kökdener and Polat (2014) and Abajue *et al.* (2015). The family Carabidae was represented by *Harpalus honestus* and *Galeritiola africana.* They used the carrions as the extension of their habitat. The family Trogidae was represented by adult *Omorgus bachorum* which is similar to the observation of Vasconcelos and Araujo (2012), Pechal *et al.* (2014) and Strümpher *et al.* (2014). The family Cucujiformia was represented by *Laemophoeus fasciatus* which was collected during the postdecay stage. Rhipiceridae was represented by *Sandalus niger,* collected during the postdecay stage. They were among the predators of necrophagous species that fed on the carcass. The family Chrysomelidae was represented by *Lema dentipes* and family Curculimidae was represented by *Prosoestus* sp. Other families encountered in this study were *Gryllus bimaculatus* (cricket), *Lymantria dispar* (moth), *Lethocerus* sp., *Periplaneta* sp. (cockroach), *Araneus* sp. (spider), *Scolopendra gigantea* (centipide) and *Anadenobolus* sp. (milipide). These species did not lay eggs on the carcass but only feed on the carcass and used the carcass as an extension of their habitats. They are regarded

as predators, parasites and accidental or opportunistic arthropods in a crime scene. The earlier researchers also got similar opportunistic arthropods in carrion; Ekanem and Dike (2010) collected Orthoptera (Acrididae, Gryllidae), Dictoptera (Perisphaeridae), Arachnida (Aranaea) and Diplopoda (Juliforma). Okiwelu (2013) observed Araneidae (spider), Lepidoptera (*Danaus plexippus*) and Orthoptera (Gryllidae, *Monopsis argillacea*). Abajue and Ewuim (2016), observed Orthoptera (Gryllidae), Dictyoptera (Prygomorphidae, Mantidae) and Hemiptera (Plastaspidae, Coreidae, Tiphiidae) in their carrions.

Moreover, the comparative species abundant (richness and diversity) on the three types of death was done using the total number of arthropods collected from the carcass during the study period (Table 3.). The result revealed that the natural death had the highest arthropod richness (Margalef, 5.067503 and Menhinick, 1.138990). They had the most diverse arthropod species (Shannon-Wiener, 2.737732; Berger-Parker, 0.166166; Simpson's, 0.086336 and Mclntosh, 0.729243) and most evenly distributed species (Equitability of Pielo, 0.763978; Sheldon's, 0.429219; Hill's, 0.749599 and Heip's, 0.412911) than other types of death. Although natural death had the smallest insect sample (999); they had the greatest number of insect species (36) as shown in Table 3. This result can be attributed to the natural means of decomposition and arthropod colonization because there was neither open wound, effect of toxic chemicals, mummification, burial and other factors that could influence decomposition and arthropod colonization. In natural death, the Calliphoridae larvae fed on the carrion without the influence of any factors such as maggot mass. The dried flesh, bones and cartilages of natural death carcass were left for the Coleoptera larvae that came in succession and different species of the families were able to have access to the carrion. But in violent death, the Calliphoridae being the most abundant arthropod colonizers, ate up about 80% of the carrion (as a result of maggot masses on the open wound) thereby making other arthropods to feed only on 20% of the carrion. This possibly resulted to the shortesrt decomposition stages and few species of arthropod colonizers especially the Coleoptera species. Also the suicide death had higher arthropod visitors than violent death but less arthropod colonizers than natural death. This is because the chemical in suicide death killed all the arthropod visitors within 3 days of decomposition and these dead flies were all used in calculating arthropod diversity. Also the chemical deters insect's colonizers thereby lengthening the duration of decomposition. As a result of the chemical, the insects did not lay eggs on the suicide death carcass during the early stages of decomposition (leading to

decrease in decomposition rate as less maggots fed on the carrion) but as decomposition progressed, arthropods visited the scene and different species were opportune to visit the carrion (resulting in more number of species visitation).

Furthermore, in comparing the diversity of adult arthropod species that were collected from the reared larvae in the laboratory (Table 4.); it was observed that many arthropod species that fed on the carcass did not breed on it. The natural death had the most species diversity and most evenly distributed species. This is because natural death passed through natural processes of decomposition as discussed earlier. Decomposition was neither influenced by maggot mass in the open wound nor chemical that deters insects thus allowed different species of arthropods to visit and breed on the carcass on its appropriate successional pattern. The violent death had the highest sample size (942) and highest number of individual (sample size) (14) but was less diverse and unevenly distributed. This could also be attributed to the presence of open wounds which were site for Calliphora maggot mass hence attracting more sample size than other killing methods. Therefore, from this present research, one can deduce that the rate of decomposition, arthropod colonization and determination of PMI can be affected by the type of death or the killing method applied on the individual.

The species or victims postmortem interval was also determined using the maggot age and development; in violent death, the first adult Diptera that emerged from the open wound was *Chrysomyia albiceps* which emerged 9 days after death (Table 5). This result noted that the oldest adult larva was gotten from the open wound of the violent death. The eggs/larvae were collected on the 7th of March, 2017; it pupated on the 12th of March and emerged as adult blow fly on the 15th of March, 2017 under a fluctuating temperature and relative humidity of 23°C - 32°C and 69% – 82% respectively as shown in Table 6 and Table 7. This finding was similar to the larval stage duration for *C. albiceps* reared at 25°C which was recorded by Grassberger *et al*. (2003) (8 days) and Velez and Wolff (2008) (8–10 days). Al-Qurashi (2016) also noted that *C. albiceps* adult emergence took 16 and 6.25 days at constant temperature of 20°C and 35°C. This observation was also in line with the work of Abd-Algalil *et al.* (2017) who noted emergence of *Chrysomyia albiceps* after 14.1 days, 9.8 days and 9.2 days under a constant temperature of 25 °C, 30°C and 35 °C respectively. Al-shareef and Dafina *et al.* (2017), also used *Chrysomyia albiceps* to calculate the postmortem interval on the death case of 57-year old female murder

victim and that of a 30-year old male found on the Veles hospital outdoor, all in Republic of Macedonia. He noted that PMI was between 11 – 12 days under the temperature of 22.5 °C which was confirmed by other investigations carried out by the police.

In natural death, the first adult Diptera that emerged from the opened mouth was *Chrysomyia chloropyga* which emerged 9 days after death (Table 5). This result also suggested that in natural death, the oldest larva was gotten from the mouth. Samples of eggs and larvae were collected on the 20th of March, 2017; it pupated on the 2nd of April, 2017 and emerged as adult blow fly on 8th April, 2017 (Table 5). The life cycle of this species is not well known.

In suicide death, the first Diptera that emerged was *Lucilia sericata* which emerged after 19 days of collection and after 25 days of death. The eggs/ larvae were collected on 20th March, 2017 (after 6 days of death); it pupated on 2nd March, 2017 and it emerged on the 8th of April, 2017 (Table 5.) under a fluctuating temperature and relative humidity of 23°C - 32°C and 69% – 82% respectively as shown in Table 6 and Table 7. This observation is similar to that of Bansode *et al*. (2016), who noted the life cycle of *Lucilia cuprina* as 53.9 days, 13.5 days and 10.4 days at a constant temperature of 19°C, 27°C and 35°C respectively. However, there was 6 days delay in the colonization of arthropods and the larvae collected from the suicide carcass also had about 6 days delay when compared to the work of Bansode *et al.* (2016). These delays in colonization and emergence of the adult insect could be due to the influence of the chemical that was used in killing the rats; hence leading to error in PMI determination.

The results of present study with regards to decomposition, colonization and the species collected from the carcass follows the observations of earlier researchers in Nigeria including; Ekanem and Dike (2010), Ekrakene and IIoba (2011), Okiwelu (2013) Abajue *et al.* (2015), Abajue and Ewuim (2016) and Ado-bala *et al.* (2016). Therefore, this research gives additional information in handling cases of different types of death in Nigeria using Medico-legal forensic Entomology.

However, the present study suggests that the type of death or the killing method of individuals including human can result to error in determination of postmortem interval in the Medico-legal Forensic Entomology. The data gotten in this study gives clue to the forensic entomologist to predict the PMI when working with a case of suicide death (Chemical intoxication). When there

is observable mass of dead flies on the death scene; one should suspect suicide death and PMI should be calculated by adding about 12 – 13 days to the calculated PMI using maggot age and development.

CONCLUSION

The knowledge of insect bionomics (ecology) is important in solving cases of suspicious death using forensic entomology. The result show that determining PMI in cases involving other types of death (beyond natural death) could lead to error PMI. The case of suicide death caused by common house hold chemical is common in Nigeria. The result in this research can be a baseline record to assist the entomologist in determining PMI in such case in other to mitigate the prosecution of innocent ones when such case arises. However, the works of other branches of forensic sciences like forensic ordonthology, cyberforensic and forensic pathology could also be used in determination of PMI if such case of death due to chemical inoculation and other types of death arise to produce acceptable results.

RECOMMENDATION

There is need for Nigeria government to give forensic professionals the opportunity to join the law enforment agents to participating in the criminal justice. Finally, we hope that the results presented in this study will initiate further research on the application of entomological evidence for forensic purposes in Nigeria.

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APPENDIX

ANOVA FOR THE RAT DECOMPOSITION DURATION

Oneway

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
						Lower Bound	Upper Bound
Fresh	violent death	3	1.0000	.00000	.00000	1.0000	1.0000
	natural death	3	1.0000	.00000	.00000	1.0000	1.0000
	suicide death	3	1.0000	.00000	.00000	1.0000	1.0000
	Total	9	1.0000	.00000	.00000	1.0000	1.0000
Bloated	violent death	3	2.0000	.00000	.00000	2.0000	2.0000
	natural death	3	3.0000	.00000	.00000	3.0000	3.0000
	suicide death	3	2.0000	.00000	.00000	2.0000	2.0000
	Total	9	2.3333	.50000	.16667	1.9490	2.7177
Active	violent death	3	9.0000	3.46410	2.00000	.3947	17.6053
	natural death	3	7.0000	.00000	.00000	7.0000	7.0000
	suicide death	3	13.0000	.00000	.00000	13.0000	13.0000
	Total	9	9.6667	3.16228	1.05409	7.2359	12.0974
Postdecay	violent death	3	10.3333	1.15470	.66667	7.4649	13.2018
	natural death	3	11.0000	.00000	.00000	11.0000	11.0000
	suicide death	3	21.0000	.00000	.00000	21.0000	21.0000
	Total	9	14.1111	5.20683	1.73561	10.1088	18.1134
Skeletal	violent death	3	30.3333	8.08290	4.66667	10.2543	50.4124
	natural death	3	48.3333	11.54701	6.66667	19.6490	77.0177
	suicide death	3	60.0000	10.00000	5.77350	35.1586	84.8414
	Total	9	46.2222	15.56260	5.18753	34.2598	58.1847

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Fresh	Between Groups	.000	2	.000	.	.
	Within Groups	.000	6	.000		
	Total	.000	8			
Bloated	Between Groups	2.000	2	1.000	.	.
	Within Groups	.000	6	.000		
	Total	2.000	8			

	Between Groups	56.000	2	28.000	7.000	.027
Active	Within Groups	24.000	6	4.000		
	Total	80.000	8			
	Between Groups	214.222	2	107.111	241.000	.000
Postdecay	Within Groups	2.667	6	.444		
	Total	216.889	8			
	Between Groups	1340.222	2	670.111	6.731	.029
Skeletal	Within Groups	597.333	6	99.556		
	Total	1937.556	8			

Post Hoc Tests

Homogeneous Subsets

Active

Duncan

ANOVAdurationcomparism	N	Subset for alpha = 0.05	
		1	2
natural death	3	7.0000	
violent death	3	9.0000	
suicide death	3		13.0000
Sig.		.267	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Postdecay

Duncan

ANOVAdurationcomparism	N	Subset for alpha = 0.05	
		1	2
violent death	3	10.3333	
natural death	3	11.0000	
suicide death	3		21.0000
Sig.		.267	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Skeletal

Duncan

ANOVAdurationcomparism	N	Subset for alpha = 0.05	
		1	2

violent death	3	30.3333	
natural death	3	48.3333	48.3333
suicide death	3		60.0000
Sig.		.069	.202

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

ANOVA FOR ADULT INSECTS THAT EMERGED FROM THE REARED LARVAE.

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval fo	
						Lower Bound	Upper B
VIOLENTDEATH	Calliphoridae	3	249.0000	90.08885	52.01282	25.2069	472.793
	Sarcophagidae	3	46.6667	66.98010	38.67098	-119.7211	213.054
	Dermestidae	3	27.3333	13.57694	7.83865	-6.3937	61.0603
	Cleridae	3	3.6667	2.88675	1.66667	-3.5044	10.8378
	Total	12	81.6667	112.96607	32.61050	9.8914	153.441
NATURALDEATH	Calliphoridae	3	54.6667	44.27565	25.56256	-55.3201	164.653
	Sarcophagidae	3	30.0000	15.87451	9.16515	-9.4345	69.4345
	Dermestidae	3	52.6667	58.07179	33.52777	-91.5917	196.925
	Cleridae	3	7.6667	6.50641	3.75648	-8.4961	23.8295
	Total	12	36.2500	37.71695	10.88795	12.2858	60.2142

	Calliphoridae	3	.0000	.00000	.00000	.0000	.0000
	Sarcophagidae	3	2.0000	2.64575	1.52753	-4.5724	8.5724
SUICIDEDEATH	Dermestidae	3	12.0000	15.13275	8.73689	-25.5918	49.5918
	Cleridae	3	2.0000	2.64575	1.52753	-4.5724	8.5724
	Total	12	4.0000	8.25723	2.38366	-1.2464	9.2464

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
VIOLENTDEATH	Between Groups	114784.667	3	38261.556	11.961	.003
	Within Groups	25590.000	8	3198.750		
	Total	140374.667	11			
NATURALDEATH	Between Groups	4394.250	3	1464.750	1.041	.425
	Within Groups	11254.000	8	1406.750		
	Total	15648.250	11			
SUICIDEDEATH	Between Groups	264.000	3	88.000	1.449	.299
	Within Groups	486.000	8	60.750		
	Total	750.000	11			

Homogeneous Subsets

VIOLENTDEATH

	EMERGEDDEATHTYPES	N	Subset for alpha = 0.05	
			1	2
Duncan[a]	Cleridae	3	3.6667	
	Dermestidae	3	27.3333	
	Sarcophagidae	3	46.6667	
	Calliphoridae	3		249.0000
	Sig.		.398	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

NATURALDEATH

	EMERGEDDEATHTYPES	N	Subset for alpha = 0.05

			1
Duncan[a]	Cleridae	3	7.6667
	Sarcophagidae	3	30.0000
	Dermestidae	3	52.6667
	Calliphoridae	3	54.6667
	Sig.		.187

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

SUICIDEDEATH

	EMERGEDDEATHTYPES	N	Subset for alpha = 0.05
			1
Duncan[a]	Calliphoridae	3	.0000
	Sarcophagidae	3	2.0000
	Cleridae	3	2.0000
	Dermestidae	3	12.0000
	Sig.		.114

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

ARTHROPOD SPECIES DIVERSITY DURING DECOMPOSITION OF THE NINE RAT CARCASSES

ATHROPODE SPECIES DIVERSITY DURING DECOMPOSITION OF VIOLENT DEATH RAT CARCASSES						
Order	Family	Species	AVD	BVD	CVD	sumVD
Diptera	Calliphoridae	*Chrysomyia chloropyga*	24	51	68	143
		Chrysomyia albiceps	114	257	210	581
		Chrysomyia putoria	11	16	35	62
		Lucillia sericata		1	1	2
		Lucillia cuprina			5	5
		Phumosia imitans		15	5	20
		Bengalia peuhi	3		6	9
		Stomorhina rugosa	10	4	4	18
	Oestridae	*Oestrus ovis*	3		3	6
	Sarcophagidae	*Sarcophaga inzi*	98	21	38	157
		Sarcophaga exuberans	63	7	8	78
	Muscidae	*Musca domestica*	10		8	18

		Morellia nilotica	6	3	5	14
Coleoptera	Dermestidae	*Dermestes frichii*	6	25	13	44
		Dermestes maculates	4	13	7	24
		Dermestes atar	1	3	3	7
		Trogoderma granaries		2	5	7
	Cleridae	*Necropis rufipes*	2	2	5	9
	Histeridae	*Euspilotus assimilis*			1	1
		Euspilotus scrupularis				
		Saprinus impressus				
		Platysoma leconti				
		Margarinotus brunneus				
	Curculimidae	*Prosoestus sp.*				
	Chrysomelidae	*Lema dentipes*				
	Scarabaeidae	*Ormogus bachorum*				
		Gymnopeurus capensis				
		Gymnopeurus Laevicollis		2		2
		Gymnopeurus fulgidus				
		Phanaeus igneus				
	Carabidae	*Harpalus honestus*				
		Ataenius insulptu *inscuptus*				
		Euphoria kernii				
		Galeritiola Africana				
	Cucujiformia	*Laemophloeus fasciatus*				
Hymenoptera	Formicidae	*Oecophylla sp.*	1			1
		Camponotus perrisi	15	8	30	53
		Camponotus maculatus	33	3	63	99
		Monomorium minimum	30	25	30	85
Lepidoptera	Heterocera	*Moth*				
	Vespidae	*Vespa orientalis*				
Hemiptera	Belostomatidae	*Lethocerus sp.*				
Dictyoptera	Blattidae	*Wild cockroach*	1	5		6
Orthoptera	Acredidae	*Gryllus sp.*	4	15	7	26
Arachnida	Arachnidae	*Unidentified*			6	6
Myriapoda	Diplopoda	*Unidentified*				
	Chilopoda	*Unidentified*				

Order	Family	Species	AND	BND	CND	SumND
Diptera	Calliphoridae	*Chrysomyia chloropyga*	3	30	18	51
		Chrysomyia albiceps	15	49	10	74
		Chrysomyia putoria		6		6
		Lucillia sericata				
		Lucillia cuprina				
		Phumosia imitans				
		Bengalia peuhi		2		2
		Stomorhina rugosa	4	3		7
	Oestridae	*Oestrus ovis*	1	2	4	7
	Sarcophagidae	*Sarcophaga inzi*	36	42	38	116
		Sarcophaga exuberans	5	2	3	10
	Muscidae	*Musca domestica*	27	50	89	166
		Morellia nilotica	15	10	35	60
Coleoptera	Dermestidae	*Dermestes frichii*	10	80	21	111
		Dermestes maculates	8	44	11	63
		Dermestes atar	1	4	5	10
		Trogoderma granaries		6	3	9
	Cleridae	*Necropis rufipes*	7	15	2	24
	Histeridae	*Euspilotus assimilis*			7	7
		Euspilotus scrupularis				
		Saprinus impressus		2	1	3
		Platysoma leconti			1	1
		Margarinotus brunneus				
	Curculimidae	*Prosoestus sp.*				
	Chrysomelidae	*Lema dentipes*			1	1
	Scarabaeidae	*Ormogus bachorum*			4	4
		Gymnopeurus capensis				
		Gymnopeurus Laevicollis			7	7
		Gymnopeurus fulgidus			3	3
		Phanaeus igneus				
	Carabidae	*Harpalus honestus*				
		Ataenius insulptu			1	1
		Euphoria kernii			3	3
		Galeritiola Africana			2	2
	Cucujiformia	*Laemophloeus fasciatus*			1	1
Hymenoptera	Formicidae	*Oecophylla sp.*				
		Camponotus maculatus		44	20	64
		Camponotus perrisi	46	3	33	82
		Monomorium minimum	28	19	23	70
Lepidoptera	Heterocera	*Moth*			3	3

	Vespidae	*Vespa orientalis*			1	1
Hemiptera	Belostomatidae	*Lethocerus sp.*			2	2
Dictyoptera	Blattidae	*Wild cockroach*	1	3		4
Orthoptera	Acredidae	*Gryllus sp.*				
Arachnida	Arachnidae	*Unidentified*			16	16
Myriapoda	Diplopoda	*Unidentified*			5	5
	Chilopoda	*Unidentified*			3	3

ATHROPODE SPECIES DIVERSITY DURING DECOMPOSITION OF SIUCIDE DEATH RAT CARCASSES

Order	Family	Species	ASD	BSD	CSD	SumSD
Diptera	Calliphoridae	*Chrysomyia chloropyga*	70	200	5	275
		Chrysomyia albiceps	269	268	22	559
		Chrysomyia putoria	20	28	0	48
		Lucillia sericata				
		Lucillia cuprina				
		Phumosia imitans				
		Bengalia peuhi	9	1		10
		Stomorhina rugosa				
	Oestridae	*Oestrus ovis*				
	Sarcophagidae	*Sarcophaga inzi*	72	36	67	175
		Sarcophaga exuberans	3	5	1	9
	Muscidae	*Musca domestica*	77	10	54	141
		Morellia nilotica	27	35	25	87
Coleoptera	Dermestidae	*Dermestes frichii*	18	22		40
		Dermestes maculates	4	8		12
		Dermestes atar		1		1
		Trogoderma granaries				
	Cleridae	*Necropis rufipes*	2	8		10
	Histeridae	*Euspilotus assimilis*				
		Euspilotus scrupularis			3	3
		Saprinus impressus	4		2	6
		Platysoma leconti	1		14	15
		Margarinotus brunneus	1		1	2
	Curculimidae	*Prosoestus sp.*	1			1
	Chrysomelidae	*Lema dentipes*				
	Scarabaeidae	*Ormogus bachorum*			4	4
		Gymnopeurus capensis	2	7	2	11
		Gymnopeurus Laevicollis	3		27	30
		Gymnopeurus fulgidus			2	2
		Phanaeus igneus	2			2

	Carabidae	*Harpalus honestus*			1	1
		Ataenius insulptu			2	2
		Euphoria kernii			2	2
		Galeritiola Africana				
	Cucujiformia	*Laemophloeus fasciatus*				
Hymenoptera	Formicidae	*Oecophylla sp.*		377		377
		Camponotus maculates	10			10
		Camponotus Perrisi	668	6	11	685
		Monomorium minimum	50			50
Lepidoptera	Heterocera	*Moth*				
	Vespidae	*Vespa orientalis*			1	1
Hemiptera	Belostomatidae	*Lethocerus sp.*			2	2
Dictyoptera	Blattidae	*Wild cockroach*		1		1
Orthoptera	Acredidae	*Gryllus sp.*		5		5
Arachnida	Arachnidae	*Unidentified*		1		1
Myriapoda	Diplopoda	*Unidentified*				
	Chilopoda	*Unidentified*		1	1	2

			Simp		Shan-W				mod Shan-W	
Species	sumVD	pi	pi2	Ln(Pi)	piLn(pi)	1-pi	(1-pi)^n	1-(1-pi)^n	divid	ni^2
Chrysomyia chloropyga	143	0.096426	0.009298	-2.33898	-0.22554	0.903574	4.94E-66	1	-0.22554	20449
Chrysomyia albiceps	581	0.391773	0.153486	-0.93707	-0.36712	0.608227	4.94E-66	1	-0.36712	337561
Chrysomyia putoria	62	0.041807	0.001748	-3.17469	-0.13272	0.958193	4.94E-66	1	-0.13272	3844
Lucillia sericata	2	0.001349	1.82E-06	-6.60868	-0.00891	0.998651	4.94E-66	1	-0.00891	4
Lucillia cuprina	5	0.003372	1.14E-05	-5.69238	-0.01919	0.996628	4.94E-66	1	-0.01919	25
Phumosia imitans	20	0.013486	0.000182	-4.30609	-0.05807	0.986514	4.94E-66	1	-0.05807	400
Bengalia peuhi	9	0.006069	3.68E-05	-5.1046	-0.03098	0.993931	4.94E-66	1	-0.03098	81
Stomorhina rugosa	18	0.012138	0.000147	-4.41145	-0.05354	0.987862	4.94E-66	1	-0.05354	324
Oestrus ovis	6	0.004046	1.64E-05	-5.51006	-0.02229	0.995954	4.94E-66	1	-0.02229	36
Sarcophaga inzi	157	0.105866	0.011208	-2.24558	-0.23773	0.894134	4.94E-66	1	-0.23773	24649
Sarcophaga exuberans	78	0.052596	0.002766	-2.94511	-0.1549	0.947404	4.94E-66	1	-0.1549	6084
Musca domestica	18	0.012138	0.000147	-4.41145	-0.05354	0.987862	4.94E-66	1	-0.05354	324
Morellia nilotica	14	0.00944	8.91E-05	-4.66277	-0.04402	0.99056	4.94E-66	1	-0.04402	196
Dermestes frichii	44	0.02967	0.00088	-3.51763	-0.10437	0.97033	4.94E-66	1	-0.10437	1936
Dermestes maculates	24	0.016183	0.000262	-4.12377	-0.06674	0.983817	4.94E-66	1	-0.06674	576
Dermestes atar	7	0.00472	2.23E-05	-5.35591	-0.02528	0.99528	4.94E-66	1	-0.02528	49
Trogoderma granarius	7	0.00472	2.23E-05	-5.35591	-0.02528	0.99528	4.94E-66	1	-0.02528	49
Necropis rufipes	9	0.006069	3.68E-05	-5.1046	-0.03098	0.993931	4.94E-66	1	-0.03098	81
Euspilotus assimilis	1	0.000674	4.55E-07	-7.30182	-0.00492	0.999326	4.94E-66	1	-0.00492	1
Euspilotus scrupularis										

Saprinus impressus										
Platysoma leconti										
Margarinotus brunneus										
Prosoestus sp.										
Lema dentipes										
Ormogus bachorum										
Gymnopeurus capensis										
Gymnopeurus Laevicollis	2	0.001349	1.82E-06	-6.60868	-0.00891	0.998651	4.94E-66	1	-0.00891	4
Gymnopeurus fulgidus										
Phanaeus igneus										
Harpalus honestus										
Ataenius insulptu										
Euphoria kernii										
Galeritiola africana										
Laemophloeus fasciatus										
Oecophylla sp.	1	0.000674	4.55E-07	-7.30182	-0.00492	0.999326	4.94E-66	1	-0.00492	1
Camponotus maculates	53	0.035738	0.001277	-3.33153	-0.11906	0.964262	4.94E-66	1	-0.11906	2809
Camponotus perrisi	99	0.066757	0.004456	-2.7067	-0.18069	0.933243	4.94E-66	1	-0.18069	9801
Monomorium minimum	85	0.057316	0.003285	-2.85917	-0.16388	0.942684	4.94E-66	1	-0.16388	7225
Moth										
Vespa orientalis										
Lethocerus sp.										
Wild cockroach	6	0.004046	1.64E-05	-5.51006	-0.02229	0.995954	4.94E-66	1	-0.02229	36
Gryllus sp.	26	0.017532	0.000307	-4.04373	-0.07089	0.982468	4.94E-66	1	-0.07089	676
Unidentified	6	0.004046	1.64E-05	-5.51006	-0.02229	0.995954	4.94E-66	1	-0.02229	36
Unidentified										
Unidentified										
Sum	1483	1	0.189724		-2.25909				-2.25909	
n – 1	1482				2.259086				2.259086	
n(n-1)	2197806									417257
N0	27								U	645.9543
	Gini-simpson		0.810276						N-U	837.0457
	Mod Simp (1/D)		5.270826						N-sqrtN	1444.49
	N1		9.574319					McIntosh	D	0.579475
	evennes Simp		0.195216							
	Pielo		0.685436							
	Sheldon's		0.354604							
	Heip's		0.329782							
	Hill's		0.550517							

	Mod Hill's		0.498095							
	richness index									
	Margalef		3.560755							
	Menhinick		0.701121							
	Berger-Parker	0.391773								

		Simp		Shan-W				mod Shan-W	
SumND	pi	pi2	Ln(Pi)	piLn(pi)	1-pi	(1-pi)^n	1-(1-pi)^n	divid	ni^2
51	0.051051	0.002606	-2.97493	-0.15187	0.948949	1.84E-23	1	-0.15187	2601
74	0.074074	0.005487	-2.60269	-0.19279	0.925926	1.84E-23	1	-0.19279	5476
6	0.006006	3.61E-05	-5.115	-0.03072	0.993994	1.84E-23	1	-0.03072	36
2	0.002002	4.01E-06	-6.21361	-0.01244	0.997998	1.84E-23	1	-0.01244	4
7	0.007007	4.91E-05	-4.96084	-0.03476	0.992993	1.84E-23	1	-0.03476	49
7	0.007007	4.91E-05	-4.96084	-0.03476	0.992993	1.84E-23	1	-0.03476	49
116	0.116116	0.013483	-2.15316	-0.25002	0.883884	1.84E-23	1	-0.25002	13456
10	0.01001	0.0001	-4.60417	-0.04609	0.98999	1.84E-23	1	-0.04609	100
166	0.166166	0.027611	-1.79477	-0.29823	0.833834	1.84E-23	1	-0.29823	27556
60	0.06006	0.003607	-2.81241	-0.16891	0.93994	1.84E-23	1	-0.16891	3600
111	0.111111	0.012346	-2.19722	-0.24414	0.888889	1.84E-23	1	-0.24414	12321
63	0.063063	0.003977	-2.76362	-0.17428	0.936937	1.84E-23	1	-0.17428	3969
10	0.01001	0.0001	-4.60417	-0.04609	0.98999	1.84E-23	1	-0.04609	100
9	0.009009	8.12E-05	-4.70953	-0.04243	0.990991	1.84E-23	1	-0.04243	81
24	0.024024	0.000577	-3.7287	-0.08958	0.975976	1.84E-23	1	-0.08958	576
7	0.007007	4.91E-05	-4.96084	-0.03476	0.992993	1.84E-23	1	-0.03476	49
3	0.003003	9.02E-06	-5.80814	-0.01744	0.996997	1.84E-23	1	-0.01744	9
1	0.001001	1E-06	-6.90675	-0.00691	0.998999	1.84E-23	1	-0.00691	1
1	0.001001	1E-06	-6.90675	-0.00691	0.998999	1.84E-23	1	-0.00691	1
4	0.004004	1.6E-05	-5.52046	-0.0221	0.995996	1.84E-23	1	-0.0221	16
7	0.007007	4.91E-05	-4.96084	-0.03476	0.992993	1.84E-23	1	-0.03476	49

3	0.003003	9.02E-06	-5.80814	-0.01744	0.996997	1.84E-23	1	-0.01744	9
1	0.001001	1E-06	-6.90675	-0.00691	0.998999	1.84E-23	1	-0.00691	1
3	0.003003	9.02E-06	-5.80814	-0.01744	0.996997	1.84E-23	1	-0.01744	9
2	0.002002	4.01E-06	-6.21361	-0.01244	0.997998	1.84E-23	1	-0.01244	4
1	0.001001	1E-06	-6.90675	-0.00691	0.998999	1.84E-23	1	-0.00691	1
64	0.064064	0.004104	-2.74787	-0.17604	0.935936	1.84E-23	1	-0.17604	4096
82	0.082082	0.006737	-2.50004	-0.20521	0.917918	1.84E-23	1	-0.20521	6724
70	0.07007	0.00491	-2.65826	-0.18626	0.92993	1.84E-23	1	-0.18626	4900
3	0.003003	9.02E-06	-5.80814	-0.01744	0.996997	1.84E-23	1	-0.01744	9
1	0.001001	1E-06	-6.90675	-0.00691	0.998999	1.84E-23	1	-0.00691	1
2	0.002002	4.01E-06	-6.21361	-0.01244	0.997998	1.84E-23	1	-0.01244	4
4	0.004004	1.6E-05	-5.52046	-0.0221	0.995996	1.84E-23	1	-0.0221	16
16	0.016016	0.000257	-4.13417	-0.06621	0.983984	1.84E-23	1	-0.06621	256
5	0.005005	2.51E-05	-5.29732	-0.02651	0.994995	1.84E-23	1	-0.02651	25
3	0.003003	9.02E-06	-5.80814	-0.01744	0.996997	1.84E-23	1	-0.01744	9
999	1	0.086336		-2.73773				-2.73773	
998				2.737732				2.737732	
997002									86163
36								U	293.5353
Gini-simpson		0.913664						N-U	705.4647
Mod Simp (1/D)		11.58271						N-sqrtN	967.393
N1		15.45187					McIntosh	D	0.729243
evennes Simp		0.321742							
Pielo		0.763978							
Sheldon's		0.429219							
Heip's		0.412911							
Hill's		0.749599							
Mod Hill's		0.732272							
richness index									
Margalef		5.067503							
Menhinick		1.13899							
Berger-Parker	0.166166								

		Simp		Shan-W				mod Shan-W	
sumSD	pi	pi2	Ln(Pi)	piLn(pi)	1-pi	(1-pi)^n	1-(1-pi)^n	divid	ni^2
275	0.106507	0.011344	-2.23955	-0.23853	0.893493	5.2E-127	1	-0.23853	75625
559	0.216499	0.046872	-1.53017	-0.33128	0.783501	5.2E-127	1	-0.33128	312481
48	0.01859	0.000346	-3.98512	-0.07408	0.98141	5.2E-127	1	-0.07408	2304
10	0.003873	1.5E-05	-5.55373	-0.02151	0.996127	5.2E-127	1	-0.02151	100
175	0.067777	0.004594	-2.69153	-0.18242	0.932223	5.2E-127	1	-0.18242	30625
9	0.003486	1.21E-05	-5.65909	-0.01973	0.996514	5.2E-127	1	-0.01973	81
141	0.054609	0.002982	-2.90756	-0.15878	0.945391	5.2E-127	1	-0.15878	19881
87	0.033695	0.001135	-3.39041	-0.11424	0.966305	5.2E-127	1	-0.11424	7569
40	0.015492	0.00024	-4.16744	-0.06456	0.984508	5.2E-127	1	-0.06456	1600
12	0.004648	2.16E-05	-5.37141	-0.02496	0.995352	5.2E-127	1	-0.02496	144
1	0.000387	1.5E-07	-7.85632	-0.00304	0.999613	5.2E-127	1	-0.00304	1
10	0.003873	1.5E-05	-5.55373	-0.02151	0.996127	5.2E-127	1	-0.02151	100
3	0.001162	1.35E-06	-6.75771	-0.00785	0.998838	5.2E-127	1	-0.00785	9
6	0.002324	5.4E-06	-6.06456	-0.01409	0.997676	5.2E-127	1	-0.01409	36
15	0.005809	3.37E-05	-5.14827	-0.02991	0.994191	5.2E-127	1	-0.02991	225
2	0.000775	6E-07	-7.16317	-0.00555	0.999225	5.2E-127	1	-0.00555	4
1	0.000387	1.5E-07	-7.85632	-0.00304	0.999613	5.2E-127	1	-0.00304	1
4	0.001549	2.4E-06	-6.47003	-0.01002	0.998451	5.2E-127	1	-0.01002	16
11	0.00426	1.81E-05	-5.45842	-0.02325	0.99574	5.2E-127	1	-0.02325	121
30	0.011619	0.000135	-4.45512	-0.05176	0.988381	5.2E-127	1	-0.05176	900
2	0.000775	6E-07	-7.16317	-0.00555	0.999225	5.2E-127	1	-0.00555	4
2	0.000775	6E-07	-7.16317	-0.00555	0.999225	5.2E-127	1	-0.00555	4
1	0.000387	1.5E-07	-7.85632	-0.00304	0.999613	5.2E-127	1	-0.00304	1
2	0.000775	6E-07	-7.16317	-0.00555	0.999225	5.2E-127	1	-0.00555	4
2	0.000775	6E-07	-7.16317	-0.00555	0.999225	5.2E-127	1	-0.00555	4
377	0.146011	0.021319	-1.92407	-0.28094	0.853989	5.2E-127	1	-0.28094	142129

10	0.003873	1.5E-05	-5.55373	-0.02151	0.996127	5.2E-127	1	-0.02151	100
685	0.265298	0.070383	-1.3269	-0.35202	0.734702	5.2E-127	1	-0.35202	469225
50	0.019365	0.000375	-3.9443	-0.07638	0.980635	5.2E-127	1	-0.07638	2500
1	0.000387	1.5E-07	-7.85632	-0.00304	0.999613	5.2E-127	1	-0.00304	1
2	0.000775	6E-07	-7.16317	-0.00555	0.999225	5.2E-127	1	-0.00555	4
1	0.000387	1.5E-07	-7.85632	-0.00304	0.999613	5.2E-127	1	-0.00304	1
5	0.001936	3.75E-06	-6.24688	-0.0121	0.998064	5.2E-127	1	-0.0121	25
1	0.000387	1.5E-07	-7.85632	-0.00304	0.999613	5.2E-127	1	-0.00304	1
2	0.000775	6E-07	-7.16317	-0.00555	0.999225	5.2E-127	1	-0.00555	4
2582	1	0.159873		-2.18854				-2.18854	
2581				2.18854				2.18854	
6664142									1065830
35								U	1032.39
Gini-simpson		0.840127						N-U	1549.61
Mod Simp (1/D)		6.25496						N-sqrtN	2531.187
N1		8.922166					McIntosh	D	0.612207
evennes Simp		0.178713							
Pielo		0.615562							
Sheldon's		0.254919							
Heip's		0.233005							
Hill's		0.701058							
Mod Hill's		0.663324							
richness index									
Margalef		4.327726							
Menhinick		0.688795							
Berger-Parker	0.265298								

TEMPERATURE READING

Descriptive Statistics

	N	Minimum	Maximum	Mean	
	Statistic	Statistic	Statistic	Statistic	Std. Error
violentdeath	50	25	37	30.80	.385
naturaldeath	50	24	35	30.42	.304
suicidedeath	62	24	39	31.94	.461
Valid N (listwise)	50				

RELATIVE HUMIDITY OF THE THREE SITES AND THE LABORATORY

Descriptive Statistics

	N	Minimum	Maximum	Mean	
	Statistic	Statistic	Statistic	Statistic	Std. Error
RHsiteA	20	56	80	70.35	1.508
RHsiteB	20	69	80	75.00	.811
RHsiteC	20	57	80	69.70	1.496
Laboratory	20	69	82	74.65	.862
Valid N (listwise)	20				