CHAPTER ONE

1.0 INTRODUCTION

The study of insects has gained prominence in many researches relating to agriculture, ecology, genetics, medicine, veterinary and forensic science. The study of insects in relation to forensic science is called *forensic entomology*.

Hence, forensic entomology is the use of the knowledge of insects in investigations relating to crimes or even civil disputes. It also includes the study of other arthropods to legal issues, especially in a court of law (Okiwelu *et al*., 2008). The study has been useful in investigation of crime scenes on land and water (Keiper and Casamatta, 2001; Hobischak and Anderson, 2002). It has also evolved into *entomotoxicology*, which is a novel approach in forensic toxicology that alternatively carries out analysis of toxins on insects that have fed on decomposing cadavers. The use of insects in forensic interpretation also evaluates the microclimatic variables at crime scenes, in order to elucidate how the variations, affect the presence and development of insects with a view to estimating when death occurred.

The above empirical study has gained acceptance in the legal systems of the developed countries with many case studies. Apparently, its absence in Nigeria can be attributed to the dearth of knowledge of our legal system, due to the inability of her entomologists for now, to put up strong case that insects found on decomposing cadaver/corpse have strong roles to play in cases relating to questionable deaths. Hence, interpreting a crime scene to estimate the time of death of a body and possibly detect if poison was ingested prior to death is very crucial. This challenging aspect of forensic science in Nigerian legal system is a gap in forensic research.

1.2 Justification of Study

Determining the time of any questionable death of an individual is a critical component of forensic investigation, even when the cadaver has stayed for days or weeks and infested with insects due to decomposition. Interestingly, the chronology of such cadaveric insects do leave telltale to Entomologists to ascertain when the victim died. Such entomological tool is useful in forensic investigation of homicides and has been utilized extensively in the legal systems of the developed world. However, there is paucity of information about the use of insects in forensic investigation and toxicology in Nigeria, despite the incessant homicides, reported or witnessed regularly in our neighbourhoods. Hence, the need to cue in cadaveric insects from Nigeria into the global data base of forensic entomology and as well analyse the cadaveric larvae as alternative to body fluids, is needful to assist coronary investigators to unravel mysteries surrounding questionable deaths in Nigeria.

1.3 Aim and Objectives of the Study

The aim of the study is to investigate the decomposition processes of poisoned and asphyxiated pig cadavers in a building and at a forest at Nnamdi Azikiwe University, Awka in relation to their forensic and toxicological importance. Therefore, the objectives are to:

1. study insects associated with the decomposition rates of the pig cadavers;
2. evaluate how micro-environmental factors of the two environments influence the cadaver's decomposition, insects composition and succession and
3. assess how systemic poison influences the decomposition rates, insects oviposition and their larval development.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Historical Origin of Forensic Entomology

The book 'The Washing Away of the Wrongs: Forensic medicine in the thirteenth Century China written by B.E. McKnight in 1981 is one of the books of reference on the origin and application of insects study on homicide investigation. It was reported that a man had died in a rice field after being inflicted with multiple injuries. It was suspected that the injuries were caused by sharp metal object. The investigator for the homicide asked the suspects (rice farmers) to assemble their sickles on the ground. One of the sickles attracted blowflies, probably because of the traces of blood tissues on the sickle. The owner of the sickle was interrogated and he confessed to the killing. Research finding has established clearly the understanding of insect metamorphosis/life cycle, especially the flies. Thus, the understanding disproves the theory of spontaneous generation. The knowledge is then extended to forensic entomology which has been, and can be applied in law court. For instance, the French courtroom witnessed the first application of forensic entomology in 1850 (Bergeret, 1855) as cited by Amendt *et al*. (2004). In that report, mummified remains of a child were found behind a chimney during the house renovation. The insects found on that mummified corpse were evidence that the current occupants were not the culprits and were acquitted. During that time under review, forensic examiners do not have clear knowledge of insect biology rather their perceptions were based on casual observation. Yovanovich (1888) and Megin (1894) were the first forensic examiners who attempted to evaluate insect succession on corpses,

properly establishing the science of forensic entomology as reported by Amendt *et al*. (2004). Forensic entomology was later revived by researchers (Reiter and Wolleneck, 1982; 1983) and (Reiter, 1984). The beginning of the twenty-first century marked the acceptance of forensic entomology in many countries as a vital forensic tool as reported by Amendt *et al*. (2004). Hence, the acceptance of forensic entomology mainly in the United States of America and Europe where a good number of case reports were well documented and have led to many convictions, acquittals and discharge of suspects.

2.2 Insect Species Associated with Cadavers/Corpse

Insects have been known from the ancient time to colonize both invertebrates and vertebrate carrions. Casual observations of these allogenic communities reveal diverse species of insects. Until recently, when taxonomy became a core discipline which tries to identify living organisms based on morphological attributes of the specific organisms that have helped identify specific insects associating with vertebrate carrions. However, in relation to forensic science, insects mainly the fly larvae were largely ignored during autopsy as they were regarded as disgusting elements of decay (Catts and Goff, 1992).

It is noteworthy that immediately after death, insects will be attracted to the body often within minutes or hours depending on the location of the dead body and the season of the year, except if they were intentionally denied access.

Dead animals, including human corpse, begin to deteriorate minutes after death due to physiological changes which have come to a halt, thus leading to putrefication. Insects, especially flies are evidence of this deterioration as they are the first to detect the change and

arrive on the carrions. In addition, beetles arrive on the carrions few days after death to feed on the soft and dried tissues which aid to skeletonise the carrions (Abajue *et al*., 2013).

Smith (1986) categorized four carrion ecological communities as:

1. Necrophagous insects; these are insects that feed on the carrions;
2. Predators and parasites of necrophagous insects; these are insects that solely feed on other insects or arthropods on the carrion;
3. Omnivorous insects; these include insects such as wasps, ants and some beetles feeding on both the carrions and its colonizers and
4. Adventive species; such as springtail and spiders which use the corpse as an extension of their environment.

Forensic entomology utilizes insects in the first two groups. They are mainly insect species from the orders Diptera and Coleoptera. Their succession on carrions/corpses can be distinguished into different phases over the various stages of decomposition, although this has been debated (Schoenly and Reid, 1987). Nevertheless, since the attractiveness of a decaying body differs between necrophagous insects, changes over time and the colonization of the corpse will occur in a predictable sequence Amendt *et al*. (2004).

Though, there have been variou reports on the infestation time of vertebrate carrion by flies, many reports agreed that blowflies are typically the first colonizers attracted to carrions due to the odour produced during decomposition (Smith, 1986; Wall and Warnes, 1994; Fisher *et al*., 1998; Anderson, 2001), even over long distances (Braack, 1981; Erzincliogu, 1996). Olfactory stimuli, vision, colour and the presence of other conspecific in insects play role on

insects to locate dead bodies (Hall, 1995; Hall *et al*., 1995; Wall and Fisher, 2001). The presence of ammonia-rich compounds and hydrogen sulphide on the other hand are important stimulants for oviposition to take place, as well as moisture, some pheromones, and tactile stimulants (Ashwort and Wall, 1994; Fisher *et al*., 1998; Anderson, 2001). Female dipteran flies do not oviposit in dehydrated or mummified tissue as eggs and larvae need moisture for successful development (Introna and Campobasso, 2000). Hence, oviposition first occurs at the orifices or wounds on the corpse where at least the moisture content will support the eclosion of the eggs.

Interestingly, Mann *et al*. (1990) stated that not all the necrophagous insects prefer larger carcasses. Some species oviposit preferentially on smaller animals such as rodents and even snails. Several factors affect the colonization of carrions, such as its burial. The reports of Introna and Campobasso (2000) and Campobasso *et al*. (2001) stated that most dipterans may not have access to colonize carrions buried deeper than 30cm while some such as Phoridae may be found in buried coffins (Anderson, 2001). However, buried corpses decay depending on the contingencies of how it was buried (Gaudry, 2010). Literature relating to insects associating with buried corpses or carrions is lacking in Africa and Nigeria in particular, indicating that insects on buried carrions have not been explored. The taxonomic composition of African carrion communities has at least compiled three hundred species of arthropods in about forty taxonomic families, primarily of insects (Villet, 2011). These figures can be compared with the lists from the Hollarctic (Payne 1965; Smith, 1986) and the Neotropics (Carvalho and Mello-Patiu, 2008; Almeida and Mise, 2009) as reviewed by (Villet, 2011).

Nevertheless, one of the challenges of insects associated with carrions in Africa especially Nigeria is the proper identification of insects to species level based on morphological features of the adults. While very few of the identification keys can be painstakingly found, the keys to the larval stages are not found. The great challenge poses enormous task on the study as it takes for now few carrions researchers to rear the larvae to adult stage. However, this singular procedure may be hampered along the rearing process. On the contrary, developed countries in the US and UK have developed molecular methods of identification, enabling identification of all stages including eggs, thereby linking immature stages to adult identity with ease.

Reports on the references of the identified carcass arthropods and the numerical strength of specimens on them revealed that over 95% of the specimens are insects. A 65kg carcass for instance can support over 115000 maggots and 20600 histerid beetles (Braack, 1987). However, in Africa, blowfly populations are numerically dominant, but the beetles are more species-rich, and several other orders of insects may occur predictably or adventiously. This pattern is also seen in other continents, where members of the same family are often involved, suggesting that ecological principles relating to the African carrion-associating fauna may be applicable in other regions too (Villet, 2011). This is even more likely because many common carrion insect species are synanthropic and have been spread around the world by human traffic (Prins, 1979; Laurence, 1988; Braack, 1991; Williams and Villet, 2006b) and their own dispersal abilities (Povolny, 2002; Verves, 2004; Rosati and VanLaerhoven, 2007).

The diverse nature of the most characteristic carrion arthropods includes:

a. Diptera; these are dominated by blowflies (Calliphoridae) and flesh flies (Sarcophagidae). Other families include at least 14 families of flies from across the phylogenetic spectrum (Villet, 2011).
b. Coleoptera; these are beetles that constitute the group of predators and necrophages respectively on the carrions. About two dozen families of beetles have been found on carrions (Villet, 2011). About 221 species belonging to 15 families of beetles have been recorded (Almeida and Mise, 2009) as reviewed by (Villet, 2011).
c. Hymenoptera; mainly ants and wasps. Very few families have been reported to be common on decomposing carrions. Their roles on the carrions are more of ecological than forensic.
d. Other arthropods; varieties of other arthropods occur on carrions, such as myriapods and arachnids. While myriapods have been reported as adventives or incidentals, arachnids were either reported as predators, parasites or incidentals of the carrion environment (Prins, 1983; Ekanem and Dike, 2010). Other insect orders (Dictyoptera, Orthorptera, Isoptera and Hemiptera) have also been implicated on carrions in Nigeria (Ekanem and Dike, 2010; Abajue *et al*., 2013).

2.3 Carrion Infestation Time by Insects and Their Activities

Carrions or exposed corpses represent temporary food resources for varied and different community of organisms. Insects especially in the order Coleoptera and Diptera are the major constituents of the community and constitute the colonial agents saddled with the decomposition of the carrions.

Insects are attracted to a body immediately after death, often within minutes (Erzincliogu, 1983; Smith, 1986; Anderson, 2001). However, oviposition may not occur on the carrion immediately. Many taxa which appear very early at a death scene are late colonizers or even non-necrophagous species (Amendt *et al*., 2004).

Contrary reports about insects that first colonize cadavers exist. However, the contradictions may overlap as regards infestation and colonization time. Blowflies according to (Wall and Warnes, 1994; Fisher *et al*., 1998; Anderson, 2001) are typically the first colonizers attracted to the carrion by the odour produced during decomposition, even over longer distances (Braack, 1981; Erzincliogu, 1996). Reasons to justify the above claims abound in literature. According to Ashworth and Wall, (1994), Fisher *et al*. (1998) and Anderson (2001), the presence of ammonia-rich compounds and hydrogen sulphide in the carrions are important stimulants for oviposition. Moisture, pheromones and tactile stimulants are the major driving forces.

2.4 Insects Succession on Cadavers

Different stages of decomposition of cadavers or corpses have been noted with different spectra of invertebrate fauna associating with each stage and in succession though, strict adherence to the succession timetable can introduce error in estimating the PMI of a corpse (Byrd and Castner, 2010). There are considerable variabilities with respect to succession influenced by the season of the year, geographical location, body size and other factors.

Insect species associating with carrions and their times of colonization vary according to the above factors. One of the most important factors is the geographic region or the

biogeoclimatic zone, (Nyasha *et al*., 2014), which explains the habitat, vegetation, soil type and meteorological conditions of an area. These factors obviously have major influence on the insect species composition present and their seasonal availability (Anderson, 2010) as well as their diversity. Precautionary measures have been established to avoid overlap of regional interpretation. Hence, data collected for a particular region or area should be used with caution when determining the time of death in another region (Amendt *et al*., 2004). The ecology of the death scene or the degree of sun exposure is the local characteristics that can alter the pattern of carrion insects colonisation (Smith, 1986; Erzinclioglu 1996). Regarding experiments on cadaver or corpse decompositions, insects were the most important decomposition agents, being present during all the stages of decomposition. The dipterans do have a peak during the initial stages and the coleopterans during the final decomposition process (Villet, 2011).

Sequence of insects successions on cadaver or corpse decomposition agree to follow the same general patterns in both temperate and tropical areas. The succession of insect species on a carcass develops primarily as a continuum of change (Schoenly and Reid, 1987) when various stages are characterized by a different number of insect species.

Villet (2011) stated that the dynamics of carrion communities cannot be explained by a stage-based model of decomposition. There is a characteristic pattern of change that must be explained. The pattern involves initial colonization of the carrion by primary and then secondary "wet phase" necrophages, followed by their predators and parasitoids and as the soft tissues are consumed and the carrion dries out, the 'wet phase' fauna leaves and 'dry phase' necrophages become progressively and numerically dominant, until only the strict

keratophages are left to exhaust the resource. The initial influx of necrophages and their associating predators and parasitoids are rapid and there is steady and increasingly erratic attrition of the community as the carrion resource dwindles.

2.5 The Length of Decomposition Stages of Cadavers

After death, cadavers or corpses undergo natural changes through different stages of decomposition that are attractive to necrophagous insects if exposed.

Different stages of cadaver decomposition have been reported and diverse opinions exist with respect to recognizing decomposition stages. Some studies recognized five stages (Bornemissza, 1956; Payne, 1965) while Reed, (1958) recognized four stages and Fuller, (1934) recognized three stages of decomposition. Morris, (1988) recommends the Reed's classification as the most satisfactory description of decomposition stages to researchers relating to medico-legal application of entomology. Schoenly and Reid, (1987) stated that researchers must keep in mind the fact that decomposition is a continuous process and that discrete stages do not exist in nature. Some authors distinguish up to eight stages, while others stipulate only three (Coe, 1978) but with subdivisions.

Stages commonly mentioned in carrion studies a*re fresh, bloated, active decay, advanced decay and remains* (Tantawi *et al.,* 1996; Byrd and Castner, 2010; Goff, 2010). The fresh stage follows death immediately, and precedes the formation of significant amount of gas trapped within the body cavity which is characteristic of the bloated stage. Once these gases leak from the body, the carcass is *actively decaying* and is evident with insect colonization, if exposed. In *advanced decay*, fluids leak from the corpse and the remaining soft tissues

desiccate to various degrees. Rain can moisten carrion in the advanced decay stage sufficiently that it returns to the condition of active decay (Tantawi *et al.*, 1996). Remains have no soft tissues and consist of bones, teeth and keratinous materials, and may be dispersed by vertebrates (Coe, 1978). Remains cadavers weather through the effects of heat (which promotes fracturing and chemical `reactions) and moisture (which promotes weathering directly and by encouraging microbes and algae).

Observation in experimental studies shows that the stages grade into one another and some of the transitions last longer than other stages. The transitions from fresh to bloated depends on how promptly microbial activity within the body generates gas. The transition from bloated to active decay is affected by how soon insects provide vents for the gases by eating into the entrapping tissues, and is therefore associated with colonization of the internal tissues by maggots. The transition from active decay to advanced decay is usually attributed to activities of insects that perforate the body and allow fluids to drain from it, and are anecdotally ascribed to the efflux of mature maggots when they are ready to pupate. The relative lengths of the bloated and decay stages depend in part on the number of species present (Villet, 2011).

2.6 Life Cycles of Insects Associated with Cadavers

Life cycle is the predicted metamorphosis that is exhibited in the development of organism(s), it gives the general overview of the life forms of an individual, whether oviparous, viviparous, and ovoviviparous or some other forms of development.

Flies and beetles are among the insects that have life cycles which show four stages of metamorphosis. It means that each stage of the life cycle is unique and different from the

other stage. The life cycles of these insects start when the adult females lay eggs which develop to another stage called larvae. Then, the larvae will undergo developmental change to pupae and to adult stages. The estimated duration of each stage and summation of the stages from egg laying to adulthood in both flies and beetles associated with vertebrate decompositions have been documented (Reiter, 1984; Greenberg, 1991; Anderson, 2000; and Grassberger and Reiter 2002). The estimation of these stages of insects on cadavers is the primary hypothetical tool used by forensic entomologists to estimate when the animal died.

Flies associated with vertebrate cadavers tend to lay eggs in batches, and clumps of eggs are laid in places on the corpse that provide protection, moisture and food (Gennard, 2007). It was suggested that *Calliphora vicina* may lay 2000-3000 eggs in their lifetime (Hinton, 1981).

The eggs of blowflies which are the most reported egg samples recovered from vertebrate cadavers are usually very shiny and white, ranging in size from around 0.9 mm to over 1.50 mm long and 0.3-0.4 mm wide (Rognes, 1991). The emergence of the first instar larvae from the eggs called eclosion is a term also used to describe any form of hatching. However, this may not be observed in flesh fly (Sarcophagidae) which does not lay eggs on the carrion but deposit first instar larvae. This type of development has been observed in some *Calliphora vicina* fly where fertilization takes place without a suitable oviposition site available immediately (Erzinclioglu, 1996). The blowfly larvae are reported to have twelve segments. The posterior end is blunt and has two brown circular areas on the final segment; these are the posterior spiracles (Gennard, 2007). The larvae have three larval stages or instars and a particular stage is distinguishable. The specific life stage of each larva can be identified by the number of slits present in each posterior spiracle. In the first instar one slit is present, in the

second instar two slits are present and in the third instar three slits are present (Ekanem, 2000). In blowflies, there is normally a difference in size of larvae in the three larval stages. However, size is a relatively unreliable measure of age because it is dependent upon the amount and quality of food available, although a body may be considered to be abundant source of food (Gennard, 2007).

Larvae in the late third instar tend to stop feeding and become migratory, searching a drier place for pupation. This is the final developmental stage of metamorphosis into the adult stage. This is called the larval post-feeding stage. Usually, the post-feeding larva attempts to bury itself in soil or some other dark locations. They may be found by searching in the first 2-3cm depth of soil at outdoor crime scenes (Gennard, 2007). The pupa is simply the transition stage between larva and adult. It is found inside a barrel-shaped puparium, which is actually the hardened and darkened skin of the final larval instar. The puparial case changes colour overtime, becoming an oval object resembling an uncut cigar, coloured somewhere between reddish-brown and a dark mahogany brown or black (Gennard, 2007). Some attempts have been made to relate the state of colouration development of the puparium to post mortem interval but to date the method have not shown great accuracy beyond the first 24 hours (Greenberg, 1991). The emergence of the adult at the end of the life cycle is achieved by the fly pushing the cap (operculum) off the puparium, using a blood-inflated region on the head called ptilinum (Gennard, 2007). The immature stages of blowflies and other flies associated with carrion decomposition are poorly documented in comparison to the adults.

Beetles show complete metamorphosis in their development. Before reaching adulthood from the egg stage, they pass through three to five larval stages depending on species, and a pupal

stage. Beetle eggs tend to be oval, spherical or spheroid in shape and are usually considered very similar, irrespective of family or in a specially constructed chamber, when they pupate (Gennard, 2007).

The length of the life cycle will vary, depending on the family and species of beetle. While it takes days in staphylinids, the carabids take up to one year to complete one life cycle to adulthood. In some species the number of instars in the larval stage is not fixed, but is dependent on environmental conditions. Hinton (1945) reported as many as nine instars in dermestids and however, there is only one generation of beetles per year (Gennard, 2007).

The pupal duration of Dermestid species can last between 2 weeks and 2 months and that these beetles can overwinter in pupa chamber if the weather is most suitable or it is late in the season (Smith, 1986). Less detailed information is available about beetle life cycles than is known about the flies.

2.7 Toxicology Analysis of Insects Associated with Cadavers

Toxic substances in or on dead body can be bio-accumulated by feeding larvae and can affect their rate of development. The stage of insect life cycles that feeds on the cadaver is a potential reservoir of undigested flesh from the corpse. Because, in some circumstances, the flesh from the corpse can retain some type of drugs that were consumed before the person died and which may even have been the cause of death, these drugs may be recovered by analysing the insects (Introna *et al*., 1990).

Bodies in a state of advanced decomposition or that are skeletonized may be difficult to examine for toxicologically relevant substances due to lack of appropriate sources such as

tissue, blood or urine. Alternatively, analysis of the insects recovered from the body may enable toxicological assessment of the cause of death (Introna *et al.,* 2001; Campobasso *et al*., 2004). This is possible because, larvae which feed on corpse may sequester drugs and toxicants which had been ingested by the deceased person.

After maceration of the larvae, analysis such as thin-layer chromatography (TLC), radioimmunoassay (RIA), gas chromatography (GC), gas chromatography/mass spectrometry (GC/MS), or high-performance liquid chromatography/mass spectrometry (HPLC/MS) may be performed (Gagliano-Candela and Aventaggiato, 2001; Goff and Lord, 2001). This can as well be applied to adult insects or even remnants of larval and puparial shells which are often found at the death scene, even after several years (Miller *et al*., 1994; Bourel *et al*., 2001a; 2001b) as cited by Amendt *et al.* (2004).

To date, there is not much information available that indicate the role of drugs, which are present in decomposing body tissue on necrophagous larvae (Gennard, 2007). Drugs or toxicants ingested prior to death of a person may have effect on the development of larvae recovered from the decomposing body tissues. Muskaska *et al.* (2001) studied the effects of consuming liver containing either a barbiturate (sodium methohexital) or a steroid (hydrocortisone) on the development of flesh fly, *Sarcophaga tibialis* Macq. The result showed that the length of the larval stage was increased compared with the controls while the pupariation was more rapid. Arnaldos *et al*. (2005) also performed laboratory experiments to investigate the effect of heroin on the larval development of S*arcophaga tibialis* Macq and reported that the larval development was considerably longer than those larvae fed without heroin. However, Goff *et al*., (1991) reported that heroin increased the rate at which the

maggots of *Boettohersca peregrina* (Rob-Desv.) grow while increasing the duration of the pupal development. Nolte *et al*. (1992) reported that cocaine and its breakdown products have been found in small quantity in the puparium of calliphorid fly. Thus, it was evident that the drug was clearly sequestered in the larval body and retained in the next life stage.

The detection of toxicants such as mercury in the larvae of various species of blowflies reared on tissues containing known concentrations of mercury was described by Nourteva and Nourteva, (1982). In 1977 in Finland, a female corpse was found in an advanced stage of decomposition in a rural area with fly larvae infestation. The larvae were collected and reared to adult stage and analysed for mercury. The analysis of the flies shows low concentration of mercury, which suggest that the victim was from an area relatively free of mercury pollution compared to the area where the body was found. The result enabled the police to focus attention on a certain area for successful identification of the victim and resolution of the case. Hence, toxicological data from dipteran larvae seem to be reliable as well as those from cadaver tissues (Kintz *et al*., 1990). Benzodiazepines, barbiturates and tricyclic anti-depressants were detected in calliphorid larvae collected from a corpse of a 67-day postmortem (Kintz *et al.*, 1990) while Goff *et al.* (1997) analysed anti-depressant drugs from maggots and empty puparia of diptera. Therefore, ingested drugs or toxicants may influence the development of the necrophagous insects on carrions tissues. The above reports showed the potentials drugs or toxicants have on overall estimation of postmortem interval of corpse/carrion using the insects found on the decomposing body. Underestimation of the postmortem interval up to 24 hours is possible if the presence of morphine in tissue is not considered when calculating the development time in *Lucilia sericata* (Bourel *et al*., 1999).

Gunatilake and Goff (1989) concluded their report that malathion in the tissues of a decomposing body delayed insects colonization and oviposition for several days. This was contained in their study in a suicide case where the malathion pesticide had been consumed, the development stages of blowflies found on the dead body indicated a minimum postmortem interval of 5 days whilst the victim was last seen alive 8 days prior to the discovery of his body.

Paying attention to the facts known about the lifestyle of the victim may assist in interpreting the postmortem interval, using the developmental stage of the insects recovered from the body. So, all the information known about the crime scene and pre-mortem behaviour of the person should be taken into consideration when using the entomological evidence (Gennard, 2007).

Interestingly, insects collected with plant material legally prohibited can point to the part of the world the plant originated. Crosby *et al*. (1986) reported that in two separate drug seizures in New Zealand, cannabis was seized along with eight Asian species of beetles, as well as wasps and ants. The beetles were identified belonging to the families Carabidae, Bruchidae and Tenebrionidae. Considering the geographical distribution of the insects and the level of overlap of their distributions, entomologists concluded that the cannabis came from the Tenasserim region, between the Andaman Sea and Thailand. In affirmation, one of the two suspects confessed on the basis of this evidence. This information may be of forensic value to customs and excise officers (Gennard, 2007).

2.8 Micro Environmental Interactions of the Cadavers and Their Associating Insects

Micro environmental conditions are ecological variables that define a micro-habitat in terms of vegetation; soil types as well as the meteorological conditions of the area that clearly have major impact on the types and diversity of insect species present as well as their seasonal availability. Insects that are attracted to vertebrate carrions are influenced by these microenviromental variables. These variables are of vital importance when interpreting a crime scene and estimating the length of time that a body has been dead.

Insects may be present or absent on carrion depending on the microenviromental conditions of the area. Daily activity patterns in flies are influenced by seasonality and change with geographical location. Greenberg, (1990) reported that calliphorid flies do not fly in the rain. Hence, seasonal influences, such as cold and rainy weather, may inhibit or even prevent fly activity and delay oviposition (Erzinclioglu, 1996). Thus, female insects choose to lay eggs in places on a body that provide sufficient food for the new generation along with protection, moisture and a consistent microclimate for larval development. As carcass decomposes, the human corpse for instance, is large enough to support colonies of number of different species of fly (Gennard, 2007). Archer and Elgar (2003) reported that carcass after being exposed outdoors for 24 hours show that preferred colonization sites on carcass changed from the orifices to skin folds. Such locations include in between the legs or under the ear pinnae. They concluded that migration of fly larvae for instance to more favoured site was in response to food depletion.

Blowflies usually show peaks of oviposition activity in the early afternoon (Baurmgartner and Greenberg, 1987; Greenberg, 1990). This shows that light intensity and over lapping temperature and relative humidity influence oviposition in flies.

In laboratory experiments, developmental times of calliphorids were increased under a cyclical temperature regime compared to developmental times at a constant temperature (Byrd and Allen, 2001). Variation in the duratin of the life cycle occurred at higher temperatures (35 - 45°C), when adults failed to emerge and variation in the duration of the life cycle when cultures were kept at a constant temperature of 40°C or at 10°C. This observation tends to inhibit the development of *Phornia regina* when the monthly temperatures have an average below10°C.

In Afro-tropical regions and oriental regions from India to China, central South-America and Southern Europe, *Chrysomya albiceps* is commonly the initial colonizer of a corpse (Hall and Smith, 1993). It was also recorded as one of the two most encountered species in forensic cases in South Africa (Mostovski and Mansell, 2004) where it is recognized as a spring and summer species. This species was considered to fulfill the initial colonizing role played by *Calliphora* and *Lucilia* spp. in the temperate zones (Smith, 1986). *Chrysomya* species have been recorded from Northern France, Austria and in central Europe (Erzinclioglu, 2000; Grassberger *et al*., 2003). The level of interaction of these species with other species, rather than the influence of higher temperatures alone, is considered by (Grassberger *et al*., 2003) to be the important factor in determining its change in distribution.

The carrion community presents many instant changes over three time scales: circadian, annual and the duration of decomposition. Being ectothermic, most carrion animals are less active in colder conditions such as at night and in winter. The range of mechanisms affecting community dynamics over the life span of a carrion source is more complex (Villet, 2011).

The occurrence of circadian cycles in adult blowflies and flesh flies is important in forensic contexts because it constrains the hours when eggs can be laid, and this affects the estimation of postmortem intervals. If an animal dies early in the evening, flies will probably not lay eggs on it until the next day, and an estimation of the PMI based on the development of the immature flies will need to take this disparity into account (Villet 2011). Nocturnal oviposition has never been reported from outdoor sites in temperate climates, but there are several reports from tropical Asia where nocturnal temperatures are higher, (Singh and Bharti, 2001; 2008) as cited by (Villet, 2011). Circadian rhythms also affect the timing of other activities of invertebrates, such as migration of mature maggots from carrion and the eclosion of adults. Thus, Villet *et al.* (2010) suggested the effect on the accuracy of such estimates of PMIs by norming the timing of these developmental landmarks to particular times of day produces a variable bias and precision that limits the accuracy of such estimates. Similarly, seasonal cycles determine whether a species breeds on carrion at all. The core membership of the carrion community is therefore more strongly affected by a seasonal variation than by time of the day (Villet, 2011). As earlier stated, temperature truly regulates the seasonal occurrence of many carrion arthropods, producing changes in which species represent their association with one another.

Villet, (2011) stated that in South Africa, *Dermestes peruvianus* is more common in winter and *D. maculatus* in summer. *Calliphora croceipalpis* is characteristically active in winter, *Chrysomya chloropyga* in spring and *C. putoria* in later summer. In the case of the flies, these differences in seasonal occurrence are reflected in their thermophsiological tolerances and their geographical distributions (Richards *et al*., 2009a, 2009b) as cited by (Villet, 2011).

In a nutshell, the decomposition of vertebrate carrion is generally influenced by environmental conditions. This has however affected the variations in the arrival of some insect families in different geographical regions. For instance, dermestids known to inhabit dry carcasses have been reported sooner on carrions. VanLaerhoven and Arderson (1996; 1999) in North America recorded dermestids 21 days after death, when the body was reported to be in early advanced decay. Oliva (2011) in Argentina found them as early colonizers between 10 and 30 days after death. However, reports from Nigeria have reported them on day 4 (Abajue *et al*., 2013) and day 10 respectively (Ekanem and Dike, 2010; Ekrakene and Iloba, 2011). Ideally, data about insect succession on carrion for the particular region where death occurred should be used (Gernard, 2007).

2.9 Ante Mortem Insects

Insects are of reasonable value as forensic indicators in cases of neglect or abuse. Some flies are attracted to odours, such as ammonia, resulting from urine or faecal contamination. Adult flies such as greenbottle blowfly tend to be attracted to an incontinent individual; a baby that has not had its nappy changed sufficiently often or incontinent old people who have not been

assisted in maintaining their bodily hygiene (Gernard, 2007). Flies may lay their eggs in clothing or on skin. These eggs, if undiscovered, will hatch into larvae which will start feeding upon flesh or on wound, ulcers or natural entry points of the body. Over time the flesh will be eaten away and the region may be further infected by bacteria as well as being invaded by other insects. Such insect attack can also happen to animals. In particular, rabbits, pigs, dogs and sheep can be victims of fly strike because of urine or faecal material attached to their fur, fleece or hind quarters through neglect, poor caging and living conditions or ill-health reflected by scouring. Such cases are considered to be instances of physical abuse, since victims are unable to remove the eggs or maggots themselves. The result can be serious, requiring attention from veterinary surgeons and even leading to death of the animal, or requiring its euthanasia (Villet, 2011).

Care has to be taken in making assumptions about the existence of physical abuse or assault prior to death. Studies on dressed pigs showed that effect of bloat caused the same disturbance and tearing of clothes which are characteristic of sexual assault (Komar and Beattie, 1998). They considered that maggot masses were particularly important in deriving such changes to clothing. Concerted efforts have been made by developed countries of the world; especially North America and Europe to have found the above information valuable in forensic science, hence they have continued to refine the study to meet with international best practices. Africa and other developing nations, Nigeria inclusive is becoming aware of this valuable information in relation to cadaver insects. Hence, Nigeria has started collecting and studying insects of forensic importance while taking into considerations all necessary variables that may lead to bias, if questionable death case is legally presented in the country.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Site

The study sites are about 400 hundred meters away behind the Science Village, Nnamdi Azikiwe University, Awka. The study was carried out during the wet and dry seasons respectively. Awka the state capital of Anambra is located on latitude 5° and 6°25′N and longitude 7°E and 8°E. The town stretches 8 kilometer in an East-West direction along the Enugu-Onitsha expressway and about 5 kilometers in a North-South orientation. The dimension of Awka is 1,207,800m^2 or 12,007 hectares. Ecologically, Awka lies in the Guinea Savanna experiencing between 1,000 mm and 1,500mm of rain annually (Iloeje, 1981) as cited by Ewuim (2007). It also experiences two seasons; the dry and the wet seasons with a bout of harmattan between December and January. A North-South and East-West escarpment gives Awka its topographic character.

Two contrasting study sites; the premises of Department of Zoology Annex building for animal house and fish pond and a forest behind the Department of Botany garden adjacent to the animal house. The site in the Zoology Annex building is located on latitude 6°15′18.06″N and longitude 7°06′41.37″E. The forest site is located on latitude 6°15′15.32″N and longitude7°06′40.22″E. The two sites are separated by a distance of about 230 metres and are distinctively demarcated by tarred road that links Science village with Management Science, off Faculty of Social Science road (fig 3.1).

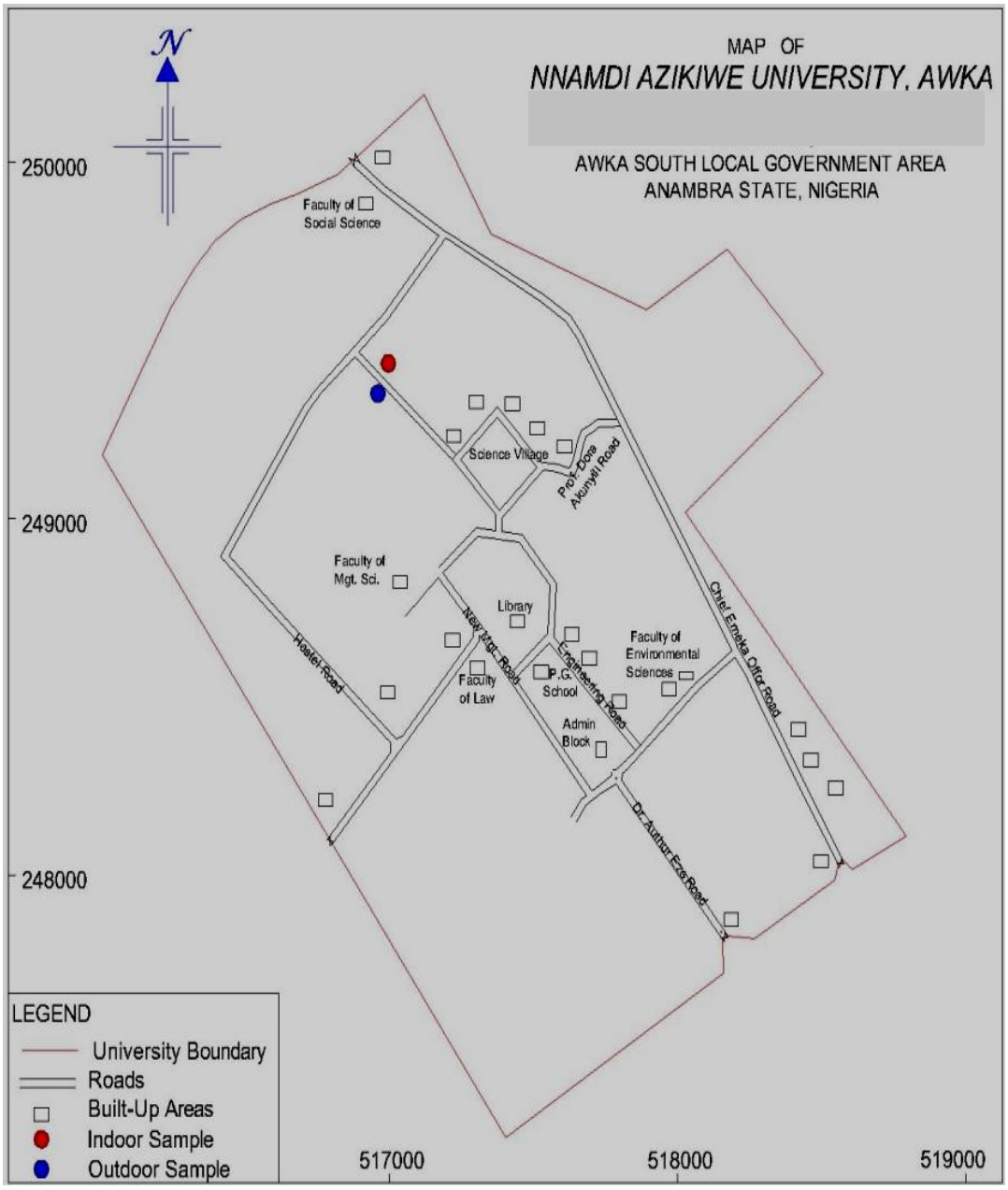


Figure 3.1: Map of Nnamdi Azikiwe University, Awka, Nigeria showing the study sites

*circular dots indicate the study sites in the map

The Zoology garden site is dominated by common weeds that include *Chromolaena odorata* (L.) R.M. King and Robinson, *Aspilia africana* (Pers.) C.D. Adams, *Abelmoschus esculentus* (L.) Moench, *Andropogan tectorum* (Schumach. and Thonn.) and *Napoleona imperialis* (P. Beauv.). Behind the animal house and adjacent to the pond is a simulated building erected to serve as indoor location for the study. The building is a wooden hut measuring 12 feet length, 10 feet wide and 10 feet height and roofed with corrugated metal sheets. The floor of the building was cemented with concrete thus, simulating a local medium room size in Awka, Anambra State, Nigeria. The building was completely enclosed but with small crevices found in between the roof and the wall as well as between the door and the wall (plate 3.1). The forest site is dominated by perennial plants and few trees. The vegetation provided shade over the floor, characterizing it with open floor peculiar to tropical forest. This site is noted as outdoor location (plate 3.2).

Plate 3.1: Overview of the Zoology animal house, where the simulated building was erected for the study

Plate 3.2: Overview of the forest, where the outdoor study was carried out

3.1.1 Study animal and killing methods

Sixteen healthy pigs, (*Sus scrofa* Linn.) with mean weight of 22.3 ± 1.9 kg, were purchased from a piggery in Okija, Anambra State for the study. They were divided into two equal groups of eight pigs. One group was sacrificed with *zinc phosphide* (rodenticicde). Each pig of the eight pigs was fed with 10 grams of the rodenticide by admixture in their food to mimic food poison. The remaining group of eight pigs was asphyxiated by strangling, to mimic 'forced' natural death by deprivation of oxygen. The dead pigs, then served as models of human corpse for the study (plate 3.3).

Plate 3.3: The experimental animal *Sus scrofa* (Linn.)

3.1.2 Experimental locations

The two experimental locations, a building (wooden hut measuring 12 feet length, 10 feet wide and 10 feet height) in the premises of Zoology Department's animal house and a forest behind the Botany Department's garden, were used to study the decomposition processes of the pig cadavers and their associated insects. Each experimental location (forest and building) received two poisoned and two asphyxiated pig cadavers simultaneously and were allowed to stand until complete skeletoniaztion was observed, during the rainy season. The pigs were deposited on a spread sacks measuring 16x8 cm^2, that serve as mats and insects collection limit. A distance of two metres between the cadavers was maintained in the building and five metres at the forest. The cadavers at the forest were protected against vertebrate scavengers with wire gauze, in the form of cage (plate 3.4). Similar process was also repeated during the dry season.

Plate 3.4: Experimental locations of the cadavers in the building and at the forest in Awka, Nigeria

3.1.3 Temperature and relative humidity data

The temperature of the cadaver, the maggot mass, and the ground surface near the cadaver were taken with bulb thermometer. The relative humidity and the ambient temperature of the study sites were taken with digital thermo-hygrometer (Mextech TM-1, model) under a shade.

3.1.4 Insect collection

Different collection approaches were employed in collecting the insects on the cadavers depending on the nature and the stage of the insects. These approaches involved the use of camel hair brush for collecting the eggs and the first larval instars of flies. Five millilitre spoon was used to collect second and third larval instars of flies while a blunt forceps was used to collect fly pupae as well as adult beetles. Insects sweep net was used to collect adult flies and other flying insects while water traps, were placed side by side on the cadavers to passively catch adult flying insects.

3.1.5 Insect rearing and collection of emerged adults

Samples of the eggs on the cadavers were collected and reared in labeled containers to adult stage in the laboratory. The rearing containers (depth 9.0 cm and mouth diameter 6.5 cm) were transparent and half-filled with a mixture of wood saw dust and sandy soil. The mixture was heated for thirty minutes in oven at 60 °C to kill microscopic organisms.

Twenty grams (20g.) of grounded *Mackerel* fish was introduced into each of the rearing containers labeled accordingly as food substrate. The eggs were then placed on the substrate so that the larvae will feed on the substrate as food when they hatch and use the mixture as a medium for pupation. Muslin cloth was used as lids for the containers, tightly held with

rubber bands. When the adult flies emerged in the containers, each of the containers was carefully placed in a larger container that was one-quarter filled with soap solution. The rubber band of the container was gently removed while the muslin cloth was still in place. The larger container was then covered with another muslin cloth and held with rubber band. Then the muslin cloth of the rearing container was carefully and gently removed with forceps. The flies then escaped into the larger container and got drowned in the soap solution. The drowned flies were collected with forceps. Larval samples collected directly from the cadavers were not reared for obvious reasons, while the dispersing larval and pupal samples found on and around the cadavers were reared without food. The emerged adults from the samples were also collected, using the same collection process.

3.1.6 Linear measurement of fly larval and pupal Samples

The length of the fly larval sample from second instar stage and pupal sample were obtained by a pair of compass and read on the metre rule. The data were tabulated based on the day of collection, the site location and from specified cadaver during both seasons.

3.1.7 Preservation and identification of insect specimens

All the insect specimens collected were temporarily preserved in 70% ethanol during the study period. At end of the study, the insects were sorted to their taxonomic group using keys provided by (Smith, 1986) and sent to a taxonomist for species identification at Insect Museum Zaria, Nigeria. The identified insects were permanently preserved in specimen bottles containing Kahle's solution.

3.2 Entomotoxicological Study

Larval and pupal samples of calliphorid flies and the larval samples of ulidiid and stratiomyiid flies collected on the poisoned and asphyxiated cadavers were assessed for zinc and phosphorus. The samples of calliphorid fly larvae were collected on day 4, 5, 6, 7, and the pupal samples collected on day 8, were used for the analysis. The larval samples of ulidiid fly collected on day 10, 11, 14, and 18 and the larval samples of stratiomyiid fly collected on day 14, 18, 22, and 28 were also used for the analysis. All the larval samples except the pupae were first killed with hot water and preserved in 80% ethanol prior to toxicological analysis.

3.2.1 Preparation of the larval samples for toxicological analysis

At the Department of Material and Energy Technology, Project Development Institute (PRODA) Enugu, Nigeria, 1g of each larval sample was washed with distilled water and placed in a beaker. Then 10 ml of 70% perchloric acid ($HOCl_4$) and 10 ml of concentrated nitric acid (HNO_3) were added in the beaker. The content of the beaker was indirectly heated in the water bath at $60^{\circ}C$ for one hour in a fume cupboard to digest the sample. After digestion, the beaker was allowed to cool. 100 ml of distilled water was added to the beaker and filtered into another beaker. (The 100 ml of distilled water was added to prevent the acids from attacking the filter paper). The filtered solution was then taken into *Atomic Absorption Spectrophotometer* (AAS– model: BUCK Scientific 210GP) flame chamber to detect and quantify zinc and phosphorus which the active ingredients of the rodenticide used as food poison to sacrifice the pigs. The process was repeated in all the samples.

3.3 Data Analysis

Analysis of variance (ANOVA) was used to determine the variations of the linear growth of the fly larvae recovered from the pig cadavers. Their mean differences were separated with least significance difference (LSD). Students't-test was used to compare the mean differences between the linear growth of the larvae recovered from the poisoned cadavers against the asphyxiated cadavers within and between the site locations, and between the two seasons.

CHAPTER FOUR

4.0 RESULTS

4.1 Decomposition of the Pig Cadavers and Their Associating Insects

During the respective studies in the building and at the forest locations, four stages of decompositions; fresh, bloated, wet decay and remains decay were observed at various durations during the rainy season (figs 4.1 and 4.2). During the dry season five stages of decomposition; fresh, bloated, wet decay, dry decay and remains were observed at various durations (figs 4.3 and 4.4).

The average daily temperature and relative humidity of the rainy season during the decomposition stages in the building were 27.5±0.40°C and 82.6±0.29 % respectively while the cadaver body's mean temperature was 30.7±0.46°C. At the forest, the average daily temperature and relative humidity were 27.2±0.29°C and 81.9±0.17 % while the cadaver body's mean temperature was 30.2±0.29°C.

The first three stages of the cadaver's decomposition, fresh, bloated and wet decay were completed in ten (10) days in the building and eleven days at the forest. The last stage of the decomposition i.e. the remains commenced on day eleven till end of the study on day one hundred and twelve (112) when insects were no longer seen.

The average daily temperature and relative humidity of the dry season recorded during the decomposition were 34.2±0.17 °C and 42.1±0.87% respectively with average cadaveric body temperature of 33.0±0.17°C. At the forest, the average daily temperature and relative humidity

were 30.8±0.29°C and 41.8±0.35% while the cadaver body's mean temperature was 41.8±0.29°C.

The first four stages of the decomposition, fresh, bloated, wet decay and dry decay were completed in thirteen (13) days at both locations. The fifth stage of the decomposition, the remains commenced on the fourteen (14) day till end of the study on day one hundred and sixteen (116), when no insects were found, coupled with the onset of rain for 2015 (table 4.1).

At the end of the study, insects collected from the decomposed cadavers were sorted and taxonomically grouped into seven orders and nineteen families. Thirty-eight species in twenty-eight genera (table 4.2). Active decomposition of the cadavers lasted ten days in the building and eleven days at the forest during the rainy season. Decomposition during the dry season lasted 14 days. The four stages of decomposition during the rainy season and the five stages during the dry season were statistically different ($P < 0.05$).

Fresh decay (rainy season: 0-1, dry season: 0-2). This stage started immediately the pigs were confirmed dead. The odour associated with decomposition was not perceived until the first sign of bloating was observed (plate 4.1). During the rainy season, insects found on the cadaver include calliphorids three minutes after the cadavers were deposited at the forest. Others include sarcophagids and formicids. Clusters of calliphorid eggs were first found on the oral region of the poisoned cadavers one hour after they were deposited at the forest. First larval instars of the calliphorid flies were observed six hours after they were deposited. No stages of insects were found in the building until five hours after they were deposited.

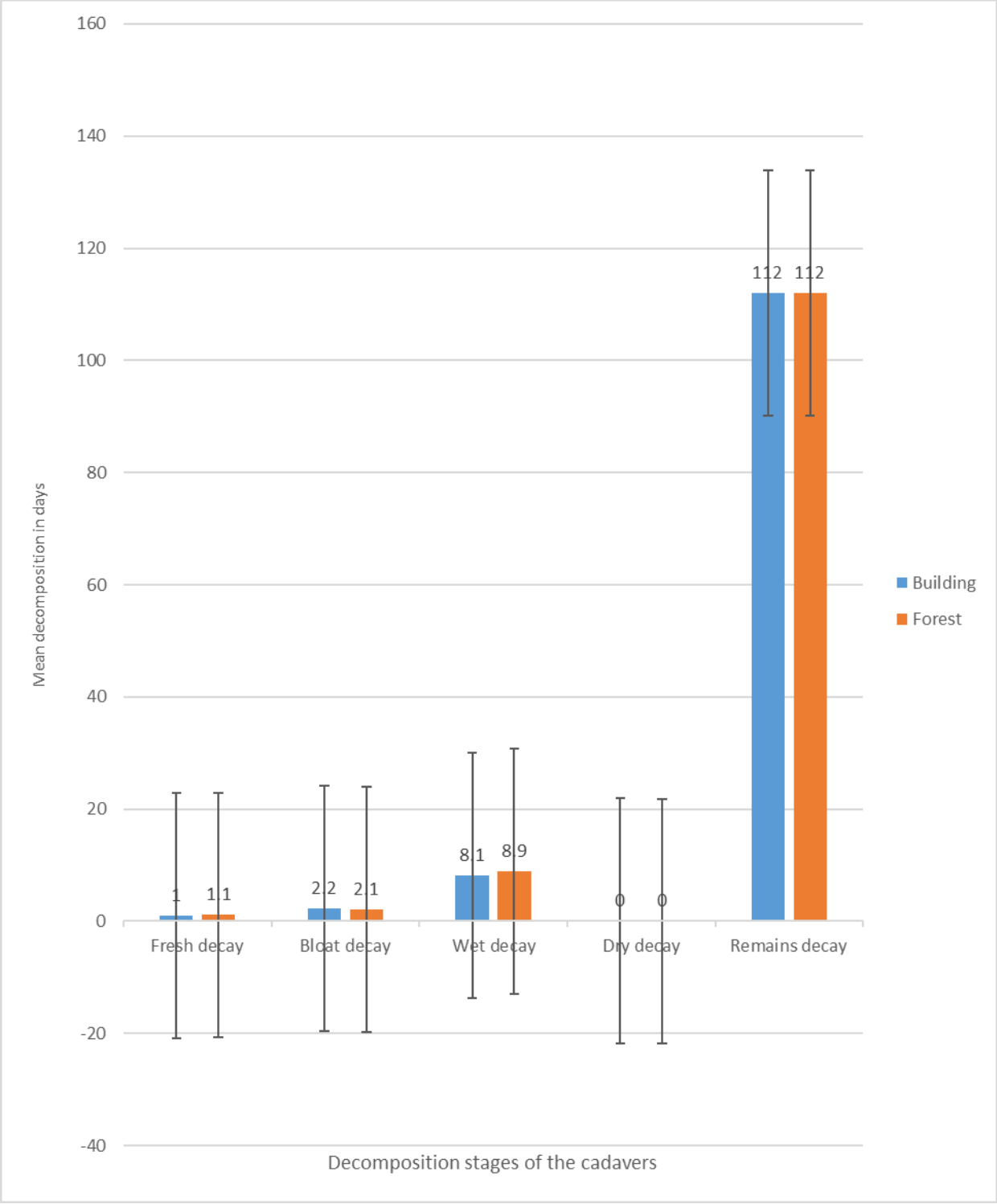


Figure 4.1: Decompsition stages of the poisoned and asphyxiated cadavers in the building and at the forest during the rainy season in Awka, Nigeria

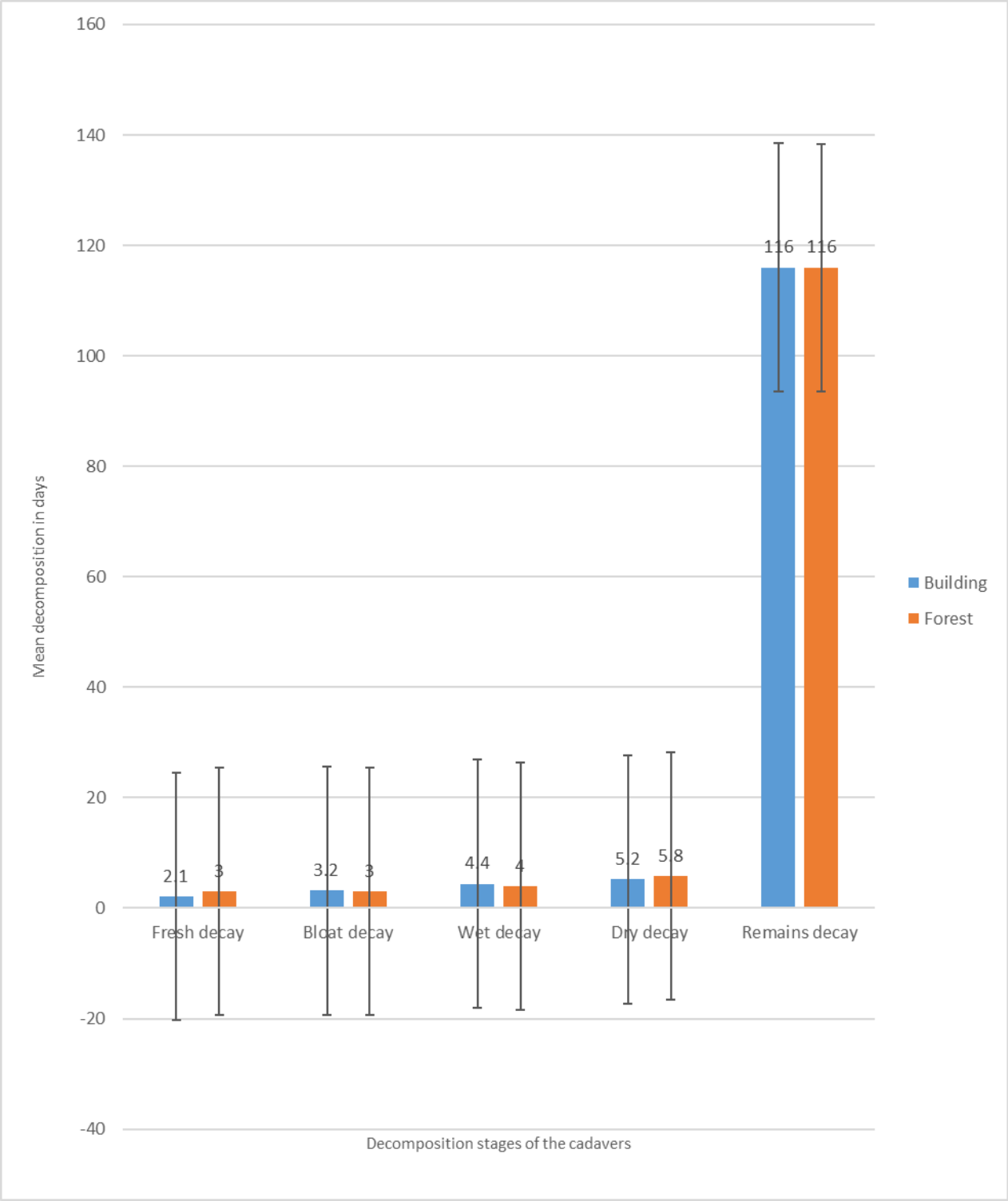


Figure 4.2: Decomposition stages of the poisoned and asphyxiated cadavers in the building and at the forest during the dry season in Awka, Nigeria d

Table 4.1: Decomposition stages of the poisoned and the asphyxiated cadavers in relation to observed air/body temperatures and relative humidity in Awka, Nigeria

Loc/season	Condition of the cadaver					Cadaver temp	Air temp	R/hum
	F	B	W	D	R	ITV (°c)	ITV (°c)	ITV (%)
Building/Rainy	0-1	2-3	3-10	0	11-112	1-5(30.7±0.46)	1-14(27.50.40)	1-14(82.6±0.29)
Forest/Rainy	0-1	1-2	3-11	0	11-112	1-5(30.2±0.29)	1-14(27.2±0.29)	1-14(81.90.17)
Building/Dry	0-2	2-4	5-9	9-13	14-116	1-10(33.0±0.17)	1-14(34.2±0.17)	1-14(42.1±0.87)
Forest/Dry	0-3	3-5	5-8	8-13	14-16	1-10(41.8±0.29)	1-14(30.8±0.29)	1-14(41.8±0.35)

F = Fresh, B = Bloated, W = Wet decay, D = Dry decay, R = Remains, ITV = Duration interval in days when the temperature and relative humidity were recorded

Table 4.2: Insects recovered from the decomposing pig cadavers in Awka, Nigeria from 2014 to 2015

Order	Family	Species
Coleoptera	Carabidae (carabids)	*Drimostoma puntulatum* (Tsch.)
	Chrysomelidae (chrysomelids)	*Buphonella* sp.
		Monolepta nigeriae (Bryant)
	Cleridae (clerids)	*Corynodes compresscornis* (Fab.)
		Necrobia ruficolis (Fab.)
		Necrobia rufipes (Deg.)
	Dermestidae (dermestids)	*Dermestes frischii* (Kug.)
	Histeridae (histerids)	*Hister monitor* (Lewis.)
		Platysoma castanipes (Mars.)
		Hister sp.
	Nitidulidae (nitidulids)	*Carpophilus dimidatus* (Fab.)
	Scarabeaidea (scarabeaids)	*Onthophagus vinctus* (Er.)
	Staphylilnidae (staphylinids)	*Philonthus* sp.
		Staphilinus sp.
	Trixagidae	Gen. nr

*Gen. nr = Genius not recognised

Table 4.2: Continued…

Order	Families	Species
Diptera	Calliphoridae (calliphorids)	*Chrysomya albiceps* (Weid.)
		Chrysomya chloropyga (Weid.)
		Chrysomya regalis (Rob-Desv.)
	Muscidae (muscids)	*Musca confusciata* (Speiser)
		Musca domestica (Linn.)
		Musca sorbens (Weid.)
		Pyrellia scientillans (Bigot)
	Sarcophagidae (sarcophagids)	*Sarcophaga inzi* (Curran)
		Sarcophaga sp.
	Stratiomyiidae (stratiomyids)	*Hermetia illucens* (Linn.)
		Sagaricera analis (Macq.)
		Sagaricera excellens (Brun)
	Ulidiidae (ulidiids)	*Chrysomyza africana* (Hendel)

Table 4.2: Continued…

Order	Families	Species
Dictyoptera	Blatoidae (blatoids)	*Blatella* sp.
Hymenoptera	Formicidae (formicids)	*Camponotus acvapimensis* (Mayr.)
		Camponotus maculatus (Fab.)
		Camponotus perrisi (For.)
		Dorylus affinis (Emy.)
		Oecophylla longinoda (Latr.)
		Pheidole sp.
Hemiptera	Lygaeidae (lygeaids)	*Oxycarenus* sp.
Homoptera	Alydidae (Alydids)	*Riptortus dentipes* (Fab.)
Orthoptera	Gryllidae (gryllids)	*Gryllus bimaculatus* (Deg.)

During the dry season, insects found were calliphorid flies after eighteen minutes of deposition. Others include sarcophagid flies, formicid ants, histerids and scarabaeids. Calliphorid eggs were found three hours after deposition on the body's interface of the cadavers at the forest. First larval instars of the calliphorid flies were observed on early morning hours of day 2. Another batch of calliphorid eggs were also observed, but now on the oral and anal surfaces of the carrions during the evening hours. No other insect groups were found until the early morning hours of day 2. Calliphorid flies and muscids were observed while the eggs were seen on the interface with the cadavers during mid afternoon.

Plate 4.1: Fresh stage of the pig cadaver immediately after death

Bloating decay (rainy season: 1-3, dry season: 2-5). This stage started at the time when the cadaveric body began to bloat due to emission of gases from the anus caused by anaerobic bacteria. The stage was easy to identify as the entire shape of the pig became spherical-like. The abdominal region first distended and then the entire body followed by swollen. The legs become rigid and stretched out while the muscles became hard. Evidence of this stage was marked by round ballon-like swellings behind the pinnae of the ears and at the anus (plate 4.2). This stage has a characteristic odour of a decayed animal matter which was easily perceived from a distance of five metres during the peak of the bloating stage.

During the rainy season, insects found on the cadavers were conspicuously dominated by clusters of calliphorids and their first larval instars. At the indoor location, the calliphorids were found with few muscids, ulidiids, stratiomyiids and formicids. In addition, few first larval instars of calliphorids and spotted number of hiserids were found.

At the outdoor location, insects found include conspicuous clusters of calliphorids at the oral and anal regions, other insects such as muscids, ulidiids, stratiomyiids were mainly hovering around the cadavers and occasionally perch either on the cadavers or on the ground while the histerid beetles and formicid ants were on the ground predating the newly hatched calliphorid larvae. At the peak of this stage, a good number of calliphorid larval mass was observed at the oral and anal regions. However, some out competed larvae from the larval mass were found wandering to other parts of the body. This behavioral shift from the orifices predisposes them as prey to the predators (formicids and histerids).

During the dry season, insects at the indoor location were calliphorid, muscids, sarcophagids, histerids, formicids, as well as dermestids. The occurrence of calliphorid larvae was preponderant at this stage.

At the outdoor location, insects also include calliphorids, sarcophagids, ulidiids, stratiomyiids, formicids, histerids, as well as staphylinids. The predating activities of the histerids and the formicid ants especially *Camponotus perrisi* were paramount. The histerids continuously took the calliphorid larvae to their tiny holes often under the cadaver body while the *C. perrisi* in their gregarious fashion continuously pick the calliphorid larvae into their nests in a reasonable distance from the cadavers (plate 4.3).

Wet decay (rainy season: 3-11, dry season: 5-9). This stage started when the body ruptured, causing the distended stomach to sharply deflate thus, forming a humid carrion island. This stage is characterised by foul odour. The orifices of the cadaver at this stage have been voraciously fed upon, making them less defined. The usual larval mass of this stage now shifts from the orifices to the armpits and in between the humerus of the legs. The stomach content was also exposed revealing the undigested food prior to death with actively feeding maggots (plate 4.4).

Plate 4.2: Bloated stage showing the bloated ballon-like swellings behind the pinnae and at the anus as evidence

Plate 4.3: *Camponotus perrisi* predating on calliphorid flies during bloated stage and returning them to distant nests

Plate 4.4: Wet decay stage of the pig cadavers evaded by larval mass of calliphorid flies

During the rainy season at the indoor location, insects were found to peak at this stage. They include calliphorid, ulidiids, muscids, stratiomyiids, histerids, dermestids, clerids, nitidulids and formicids. First larval instars of ulidiids as well as calliphorid pupae were found at this stage. It is also possible to find few empty puariae of calliphorids as a result of emergence of their first generation.

At the outdoor location, insects include calliphorids, sarcophagids, ulidiids, formicids, histerids, stratiomyiids, staphylinids, dermestids, clerids, nitidulids, chrysomelids and carabids.

During the dry season at the indoor location, insect found were calliphorids, sarcophagids, ulidiids, formicids, muscids, histerids, stratiomyiids, dermestids, clerids and nitidulids.

At the outdoor location, insects include calliphorids, sarcophagids, ulidiids, formicids, histerids, scarabaeids, stratiomyiids, staphylinids, dermestids, clerids, and nitidulids.

Dry decay (rainy season: 0, dry season: 8-13). This stage was not observed during the rainy season but was observed during the dry season at the moment when the body began to dry from the head and the surface skin began to secret oil. The original body shape partially returned with gradual reduction in size and irregular perforations at the oral orifices. The stage was characteristically marked with less foul odour but with ammonical odour. The skeletal bones, teeth and undigested stomach contents were now revealed as well as surface skin that have somehow mummified with irregular perforations. Insects found at this stage were mainly keratophages (dry skin and hair feeders) clerides and dermestids as well as omnivorous

formicids. The flies that peaked during the wet decay have all collapsed leaving behind their pupae especially the calliphorids on the interface and under the body of the cadavers.

At the indoor location during the dry season, insects found were ulidiids, formicids, histerids, stratiomyiids, dermestids, clerids, and nitidulids.

At the outdoor location during the dry season, insects found were ulidiid flies, ants, histerids, stratiomyiids, dermestids, clerids, and nitidulids (plate 4.6).

Remains (rainy season: 11-112, dry season: 14-116). This stage of decomposition was simply a skeletonised body. During the rainy season at both sites, the bones were disentangled within 20 days from their joints due to insect activities aided by frequent rainfall. However, at the indoor location, the bones were mixed up with the hairs. During the dry season, the remnants were bones, tough surface skin and hairs. At the indoor location, the bones, skin and hairs were still fed upon by the keratophages. At the outdoor location, the skin was more tough and leather-like after the first rain of the year on day 31, which washed off the hairs from the skin. At the indoor location, insects found were formicids, dermestids, clerids, nitidulids and (newly emerged calliphorids and ulidiids) and *Batrocomorphsis* sp. At the outdoor location, insects found were ants, dermestids, clerids, nitidulids and (newly emerged calliphorids and ulidiid flies) and *Batrocomorphsis* sp.

During the study, insects collected from the decomposing pig cadavers were categorized into seven orders, twenty families, twenty-eight genera and thirty-eight species. The table that shows when and where the insects were collected from the cadavers is shown in (table 4.4).

Plate 4.5: Dry decay stage of the cadaver with mass of empty puparia, clusters of calliphorid flies that newly emerged from the cadaver (evidence of dry decay or peak of wet decay) and beetles on the skeletonized body

Plate 4.6: Remains of the cadavers during seasons

Table 4.3: Decomposition stages of the cadavers and their associated insects in their order of occurrence in Awka, Nigeria

Decomposition Stages	Insects
Fresh	Calliphoridae, Sarcophagidae, Formicidae, Histeridae, Scarabaeidae
Bloated	Calliphoridae, Muscidae, Ulidiidae, Stratiomyidae, Formicidae, Histeridae, Dermestidae
Wet decay	Calliphoridae, Muscidae, Ulidiidae, Stratiomyidae, Formicidae, Histeridae, Dermestidae, Cleridae), Nitidulidae, Sarcopgagidae, Scarabaeidae, *S*taphylinidae
Dry decay	Ulidiidae, Formicidae, Histeridae, Stratiomyidae, Formicidae Dermestidae, Cleridae, Nitidulidae, (newly emerged calliphorids)
Remains	Formicidae, Dermestidae, Cleridae, Nitidulidae, Cicadellidae, (Newly emerged calliphorids and ulidiids).

Table 4.4: Insects recovered from the decomposing pig cadavers at the two contrasting sites in Awka, Nigeria from 2014 to 2015

Family	**Species**	**Building**		**Forest**	
		RNS	**DRS**	**RNS**	**DRS**
Carabidae	*Drimostoma* puntulatum (Tsch.)	X	X	#	X
Chrysomelidae	*Buphonella* sp.	X	X	#	X
	Monolepta nigeriaae (Bryant)	X	X	#	X
	*Corynodes compresscornis (*Fab.)	X	X	#	X
Cleridae	*Necrobia ruficolis* (Fab.)	#	#	#	#
	Necrobia rufipes (Deg.)	#	#	#	#
Dermestidae	*Dermestes frischii* (Kug.)	#	#	#	#
	Hister monitor (Lewis)	#	#	#	#
Histeridae	*Hister castanipe* (Mars.)	#	#	#	#
	Hister sp.	#	#	#	#
Scarabeaidae	*Onthophagus vinctus* (Er.)	X	X	#	#
Staphylinidae	*Philonthus* sp.	X	X	#	#
	Staphylinus sp.	X	X	#	#
Trixagidae	*gen.nr*	X	X	#	#

RNS; rainy season, DRS; dry season, **#;** when insect was found, X; when insect was not found

Table 4.4: Continued

Family	Species	Building		Forest	
		RNS	**DRS**	**RNS**	**DRS**
Calliphoridae	*Chrysomya albiceps* (Weid.)	#	#	#	#
	Chrysomya chloropyga (Weid.)	#	#	#	#
	Chrysomya regalis (Rob-Desv.)	#	#	#	#
Muscidae	*Musca confusciata* (Speiser)	X	X	#	X
	Musca domestica (Linn.)	#	#	#	#
	Musca sorbens (Weid.)	X	X	#	X
	Pyrellia scientillans(Bigot)	X	X	#	X
Sarcophagidae	*Sarcophaga inzi* (Curran)	#	#	#	#
	Sarcophaga sp.	X	X	#	X
Stratiomyiidae	*Hermetia illucens* (Linn.)	#	#	#	#
	Sagaricera analis (Macq.)	X	X	#	X
	Sagaricera excellens (Brun.)	X	X	#	X
Ulidiidae	*Chrysomyza africana* (Hendel)	#	#	#	#

RNS; rainy season, DRS; dry season, **#;** when insect was found, X; when insect was not found

Table 4.4: Continued

Family	Species	Building		Forest	
		RNS	DRS	RNS	DRS
Blatoidae	*Blatella* sp.	X	X	#	X
Formicidae	*Camponotus acvapimensis* (Mayr.)	X	X	#	#
	Camponotus perrisi (For.)	X	X	#	#
	Dorylus affinis (Emy.)	#	#	X	X
	Oecophylla longinoda (Latr.)	X	X	#	#
	Pheidole sp.	#	#	#	#
Lygaeidae	*Oxycarenus* sp.	#	X	X	X
Alydidae	*Riptortus dentipes* (Fab.)	X	X	X	X
Cicadellidae	*Batrocomorphis* sp.	#	#	X	#
Gryllidae	*Gryllus bimaculatus* (Deg.)	X	X	#	#

RNS; rainy season, DRS; dry season, **#;** when insect was found, X; when insect was not found

FORENSIC FLIES IN AWKA, NIGERIA

The insects below represent the pictorial view of the guilds that leave their telltale on the decomposing cadavers that enhanced the estimation of the time the pigs died.

Chrysomya chloropyga

Chrysomya regalis

Chrysomya albiceps

Chrysomyza africana

Sagaricera excellens

Hermatia illucens

Ulidiid larval mass

Calliphorid larval mass

Stratiomiid larval mass

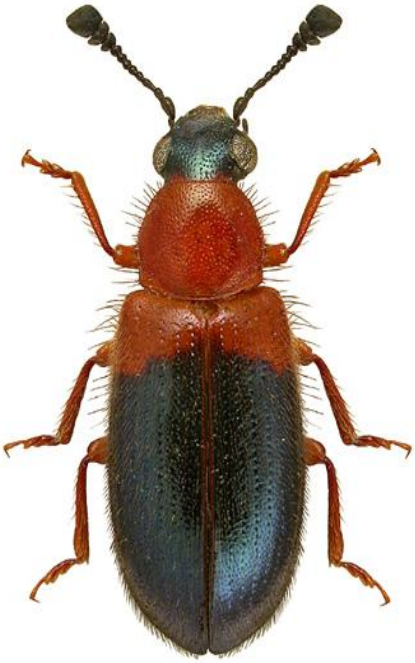
FORENSIC BEETLES IN AWKA, NIGERIA

Dermestes frischii

Dermestes larvae

Hister sp.

Necrobia rufipes



Necrobia ruficolis

Philonthus sp.

4.2. Micro-Environmental Factors of the Study Sites in Relation to the Cadaveric Fauna

The first thirty days of the study at both sites and seasons were taken as the active period of insects activities on the cadavers and were influenced by environmental factors such as temperature, relative humidity and rain. Thus, the mean daily air temperatures of the study sites and their relative humidity were taken. In addition, five days body temperatures of the cadavers were taken during the rainy season while ten days mean body temperatures of the cadavers were also taken during the dry season. The maggot mass temperatures were recorded on the cadavers from day 3 to day 6 during the rainy season at both sites and from 5 to day 9 at both sites during the dry season. The maggot mass temperatures were compared with the air temperature at both sites throughout the study period. Figures 4.3 and 4.4 show the temperatures during the rainy reason while figures 4.5 and 4.6 show the temperatures during the dry season. Figure 4.7 shows the mean temperatures of the maggot mass, the air temperatures and the relative humidity recorded during the rainy season as compared with the dry season. The results show that there was significant difference between the maggot mass and air temperatures. However, the micro-environmental variables observed at the two locations during the two seasons have no effect on the maggot mass temperature and thus, does not affect their growth irrespective of the killing methods.

It was also observed from the study that the temperature, relative humidity as well as rainfall in Awka, Nigeria have no pronounced effect on the core cadaveric insects community composition, guild structure and dynamics as shown in the succession of the insects on the cadavers at the two study locations and seasons irrespective of the sacrificing method (Figs 4.8, 4.9, 4.10 and 4.11).

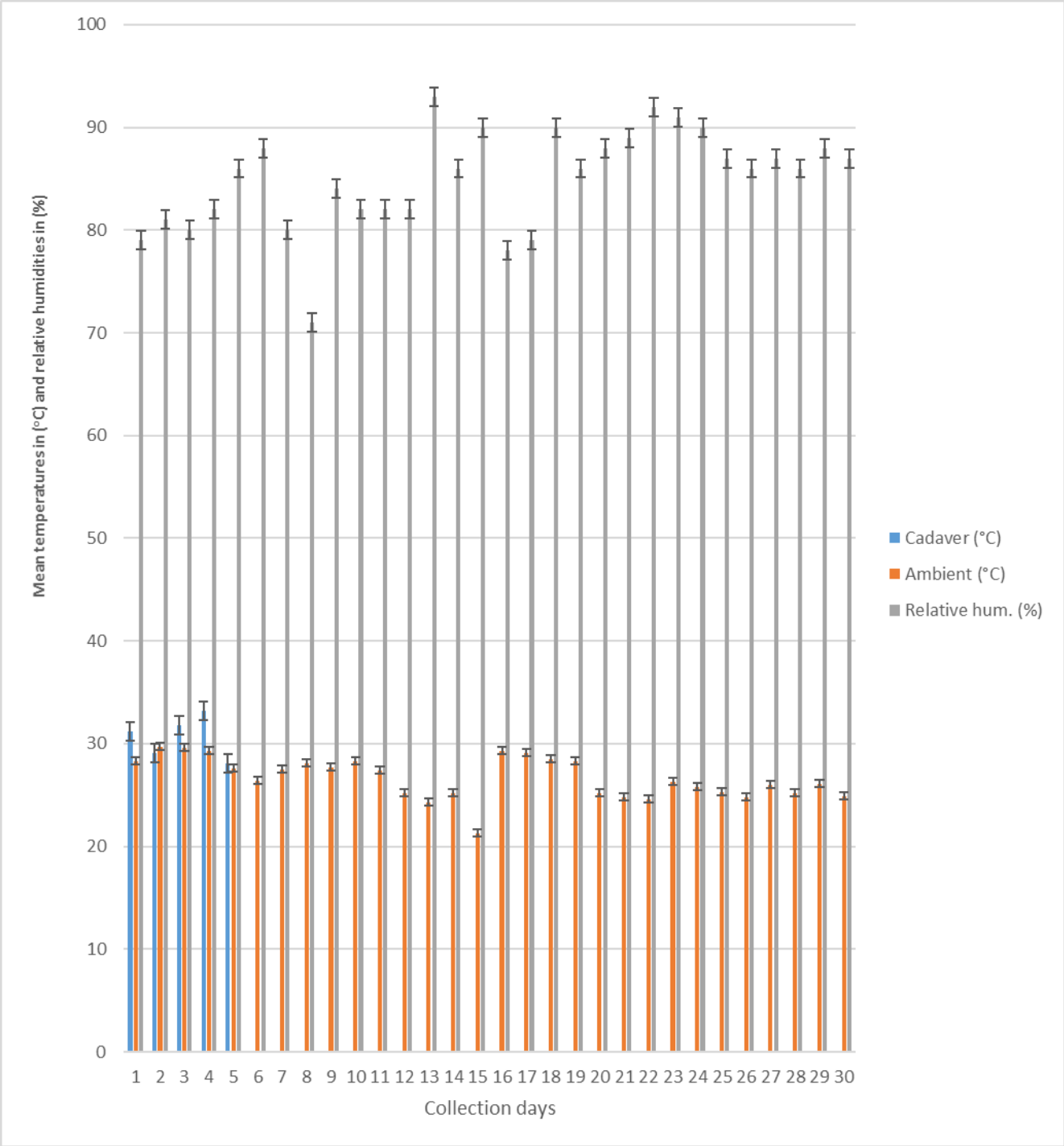


Figure 4.3: Average air and cadaveric body temperatures and relative humidity recorded during active insects activities on the cadavers during the rainy season in the building in Awka, Nigeria

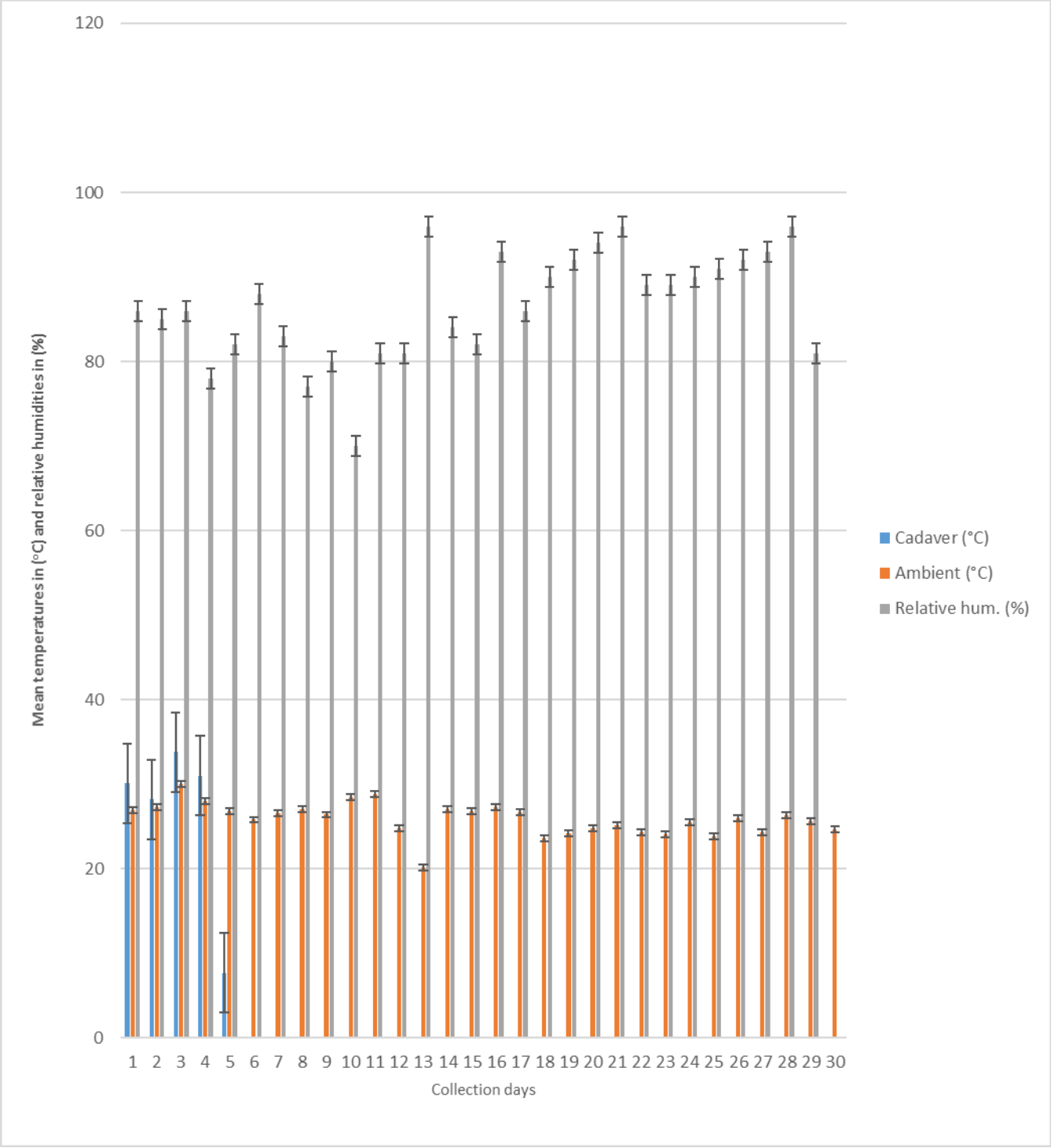


Figure 4.4: Average air and cadaveric body temperatures and relative humidity recorded during active insects activities on the cadavers during the rainy season at the forest in Awka, Nigeria

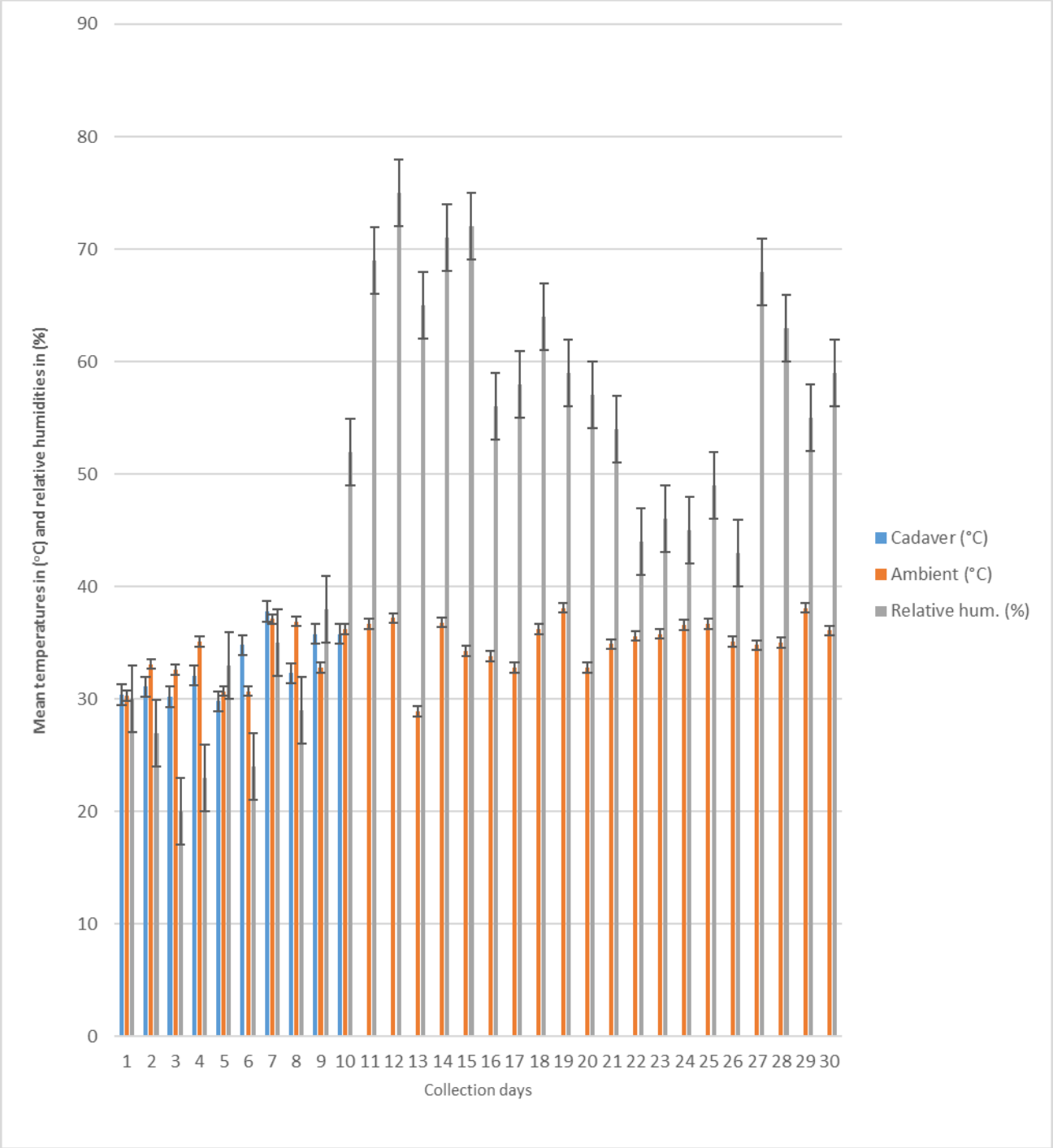


Figure 4.5: Average air and cadaver body temperatures and relative humidity recorded during active insects activities on the cadavers during the dry season in the simulated building in Awka, Nigeria

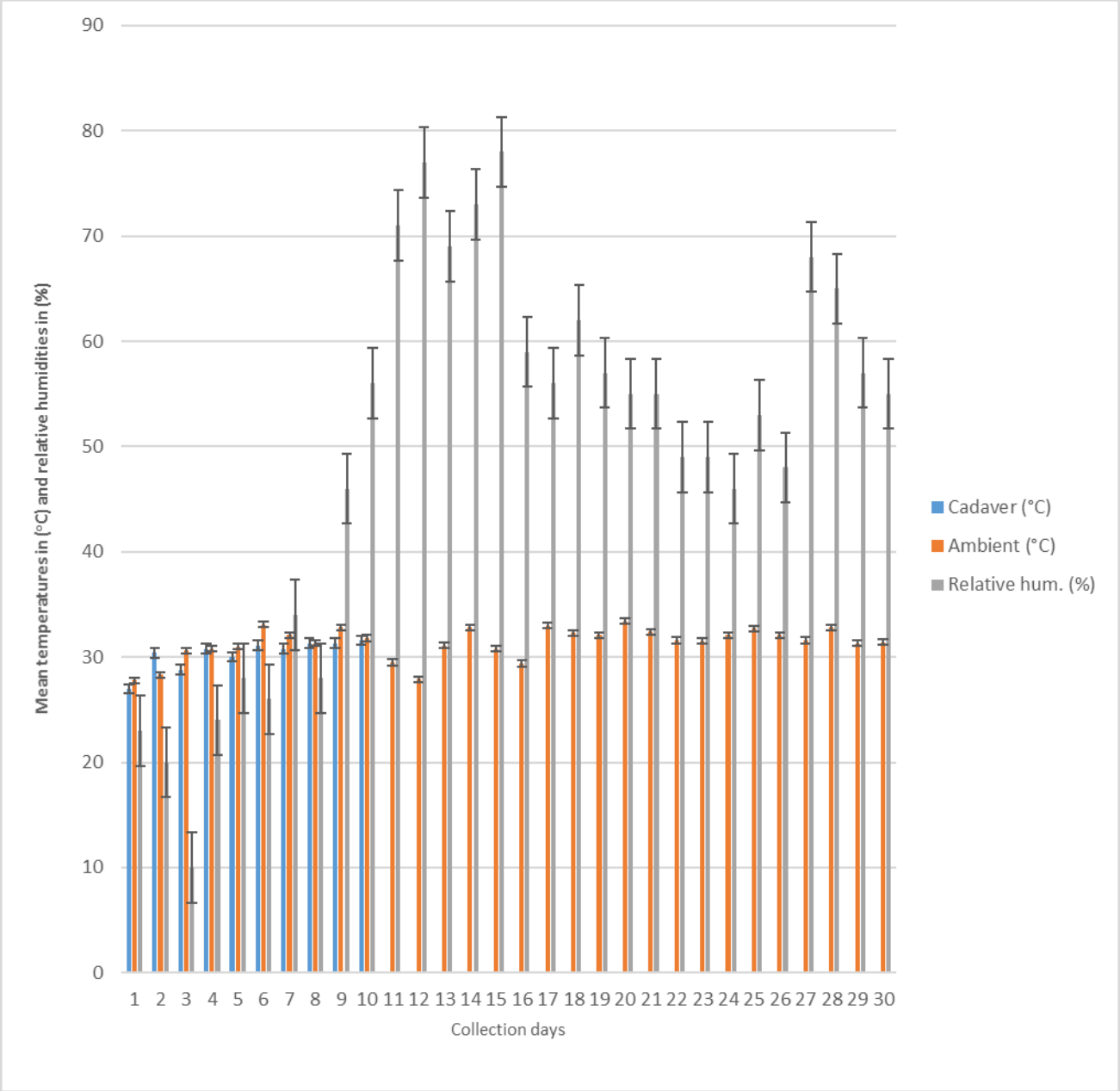


Figure 4.6: Average air and cadaver body temperatures and relative humidity recorded during active insects activities on the cadavers during the dry season at the forest in Awka, Nigeria

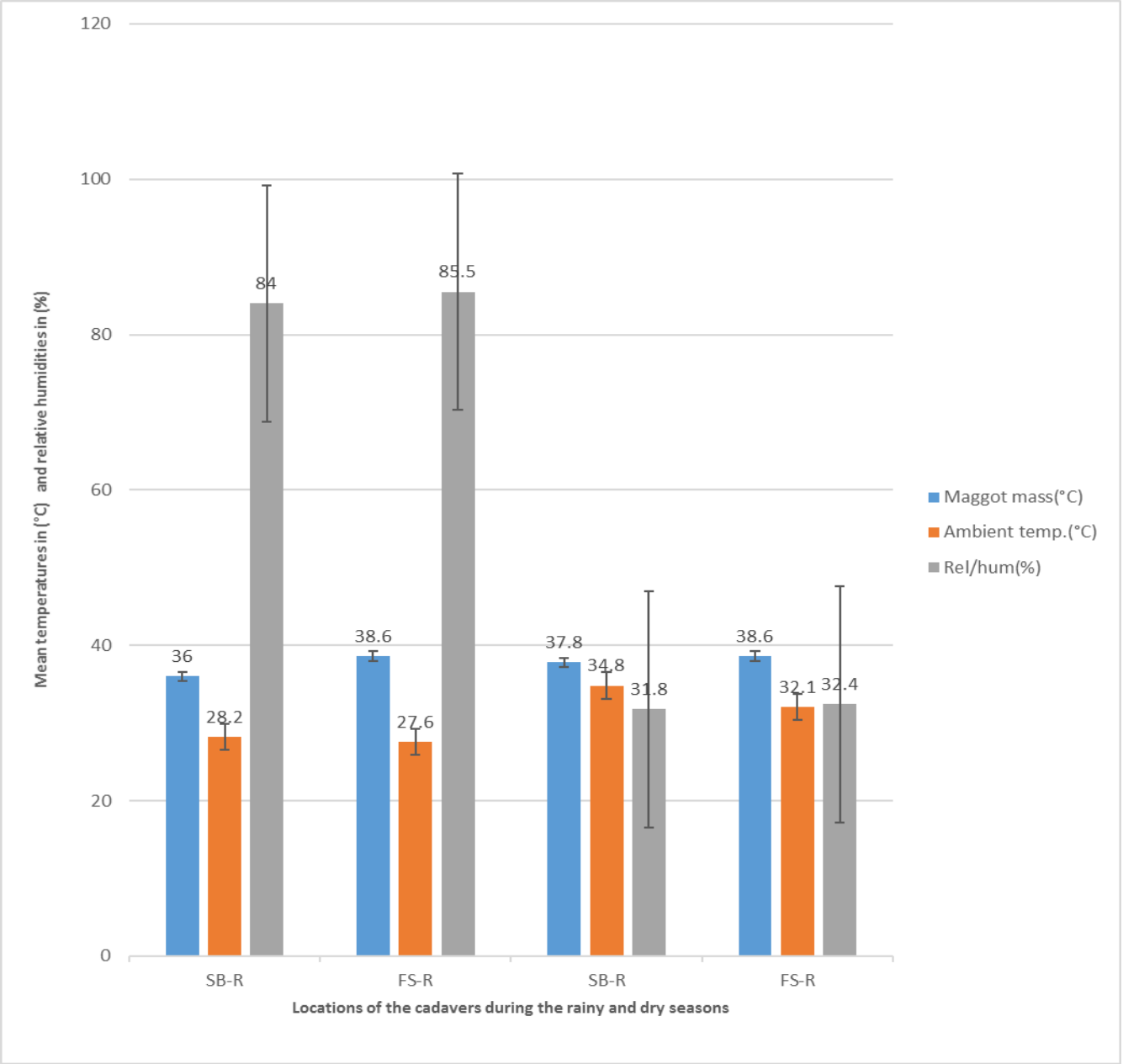


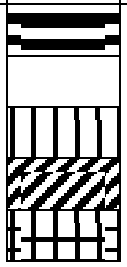
Figure 4.7: Mean temperature of maggot mass, air and relative humidity recorded for calliphorid flies between day 3 and 9 on the cadavers, during the study period in Awka, Nigeria

Note: SB-R; building during rainy season, FS-R; forest during rainy season, SB-D; building during dry season, FS-D; forest during dry season, (°C); unit of temperature

Insects																		
		Fresh			Bloated													
		1			2					3		4						
Families	Species	E	L	P	A	E	L	P	A	E	L	P	A	E	L	P		
Calliphoridae	C. albicecps																	
	C. chloropyga																	
	C. regalis																	
Muscidae	M. domestica																	
	M. confusciata																	
	M. sorbens																	
	P. scientillans																	
Stratiomyidae	H. illucens																	
	S. analis																	
	S. cellens																	
Ulidiidae	C. africana																	
Carabidae	D. puntulatum																	
Chrysomellidae	Buphonella sp.																	
Cleridae	N. ruficolis																	
	N. rufipes																	
Dermestidae	D. frischii																	
	D. maculatus																	
Histeridae	H. monitor																	
	P. castanipes																	
Nitidulidae	C. dimidatus																	
Scarabaeidae	O. vinctus																	

Staphylinidae	Philonthus sp.															
	Staphylinus sp.															
Formicidae	C. acvapimensis															
	C. maculatus															
	C. perrisi															
	D. affinis															
	O. longinoda															
	Pheidole sp.															
Alydidae	R. dentipes															

Key:



Fresh

Bloated

Wet Decay

Dry Decay

Remains

Insects																	
		Fresh				**Bloated**											
		1				**2**				**3**				**4**			
Families	**Species**	**E**	**L**	**P**	**A**	**E**	**L**	**P**	**A**	**E**	**L**	**P**	**A**	**E**	**L**	**P**	**A**
Calliphoridoe	C. albicecps																
	C. chloropyga																

	C. regalis																	
Muscidae	M. domestica																	
	M. confusciata																	
	M. sorbens																	
	P. scientillans																	
Stratiomyidae	H. illucens																	
	S. analis																	
	S. cellens																	
Ulidiidae	C. africana																	
Carabidae	D. puntulatum																	
Chrysomellidae	Buphonella sp.																	
Cleridae	N. ruficolis																	
	N. rufipes																	
Dermestidae	D. frischii																	
	D. maculatus																	
Histeridae	H. monitor																	
	P. castanipes																	
Nitidulidae	C. dimidatus																	
Scarabaeidae	O. vinctus																	
Staphylinidae	Philonthus sp.																	
	Staphylinus sp.																	
Formicidae	C. acvapimensis																	
	C. maculatus																	
	C. perrisi																	

	D. affinis																
	O. longinoda																
	Pheidole sp.																
Alydidae	R. dentipes																

Key:

Fresh
Bloated
Wet Decay
Dry Decay
Remains

Insects		Stages of Decomposition															
		Fresh				Bloated											
		1				2				3				4			
Families	Species	E	L	P	A	E	L	P	A	E	L	P	A	E	L	P	A
Calliphoridoe	C. albicecps																
	C. chloropyga																
	C. regalis																
Muscidae	M. domestica																
	M. confusciata																
	M. sorbens																
	P. scientillans																
Stratiomyidae	H. illucens																
	S. analis																
	S. cellens																
Ulidiidae	C. africana																
Carabidae	D. puntulatum																
Chrysomellidae	Buphonella sp.																
Cleridae	N. ruficolis																
	N. rufipes																
Dermestidae	D. frischii																
	D. maculatus																
Histeridae	H. monitor																
	P. castanipes																
Nitidulidae	C. dimidatus																
Scarabaeidae	O. vinctus																

Staphylinidae	Philonthus sp.																	
	Staphylinus sp.																	
Formicidae	C. acvapimensis																	
	C. maculatus																	
	C. perrisi																	
	D. affinis																	
	O. longinoda																	
	Pheidole sp.																	
Alydidae	R. dentipes																	

Key:

Fresh

Bloated

Wet Decay

Dry Decay

Remains

Insects																			
		Fresh				Bloated													
		1				2				3				4					
Families	Species	E	L	P	A	E	L	P	A	E	L	P	A	E	L	P	A		
Calliphoridoe	C. albicecps																		
	C. chloropyga																		
	C. regalis																		
Muscidae	M. domestica																		
	M. confusciata																		
	M. sorbens																		
	P. scientillans																		
Stratiomyidae	H. illucens																		
	S. analis																		
	S. cellens																		
Ulidiidae	C. africana																		
Carabidae	D. puntulatum																		
Chrysomellidae	Buphonella sp.																		
Cleridae	N. ruficolis																		
	N. rufipes																		
Dermestidae	D. frischii																		
	D. maculatus																		
Histeridae	H. monitor																		
	P. castanipes																		
Nitidulidae	C. dimidatus																		
Scarabaeidae	O. vinctus																		

Staphylinidae	Philonthus sp.																	
	Staphylinus sp.																	
Formicidae	C. acvapimensis																	
	C. maculatus																	
	C. perrisi																	
	D. affinis																	
	O. longinoda																	
	Pheidole sp.																	
Alydidae	R. dentipes																	

Key:

Fresh

Bloated

Wet Decay

Dry Decay

Remains

4.3. Evaluating the Influence of the Rodenticide (Zinc Phosphide Poison) on the Pig Cadavers and Their Associating Insects

It was also observed from the study, that the zinc phosphide rodenticide used to sacrifice the pigs had no significant effect on insects larval development and succession on the cadavers. The daily linear growths of the insect (calliphorids, ulidiids and stratiomyiids) larvae recovered from the cadavers show that growth was significant, irrespective of the sacrificing methods, site locations and the season of the study as proved by analysis of variance ($P < 0.05$). Meanwhile, students t-test showed that there was no significant growth difference ($P > 0.05$) between the mean differences of linear growth rates of larvae recovered from the asphyxiated and poisoned cadavers. Figures from 4.12 to 4.15 show the mean length of calliphorid larvae subjected to analysis of variance and 4.16 to 4.20, subjected t-test analysis. Others include figures 4.21 to 4.24 that show the mean length of ulidiid larvae subjected to analysis of variance and figures 4.25 to 4.29 subjected to t-test analysis while figures 4.30 to 4.33 showed the mean length of stratiomyiid larvae subjected to analysis of variance and figures 4.34 to 4.35 subjected to t-test analysis.

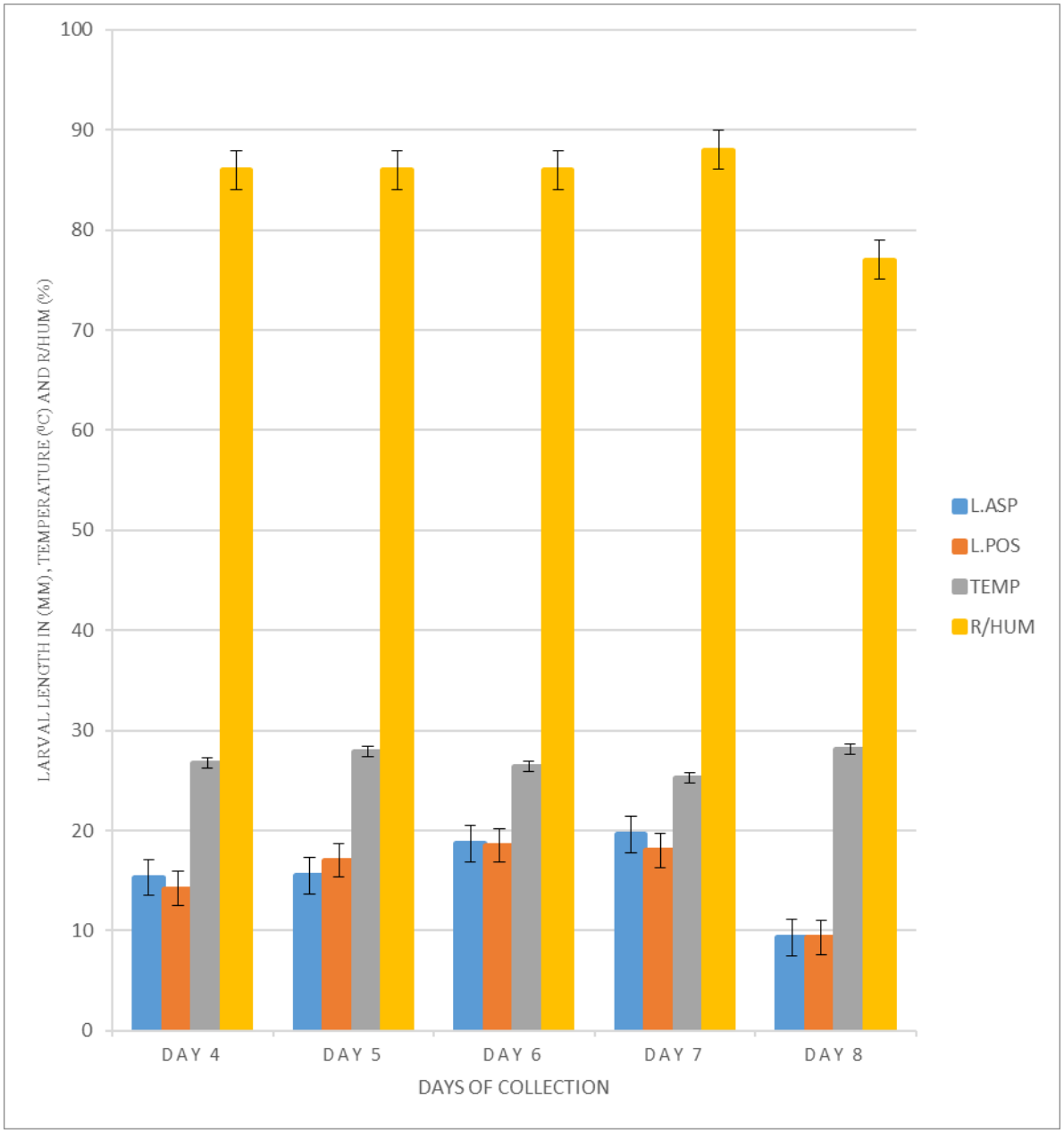


Figure 4.12: Mean length (in mm) of calliphorid larvae recovered from the poisoned and asphyxiated cadavers in the building with their temperature and relative humidity during the rainy season in Awka, Nigeria

Note: L.ASP & L.POS= larvae recovered on asphyxiated and poisoned cadavers, TEMP & R/HUM= temperature and relative humidity

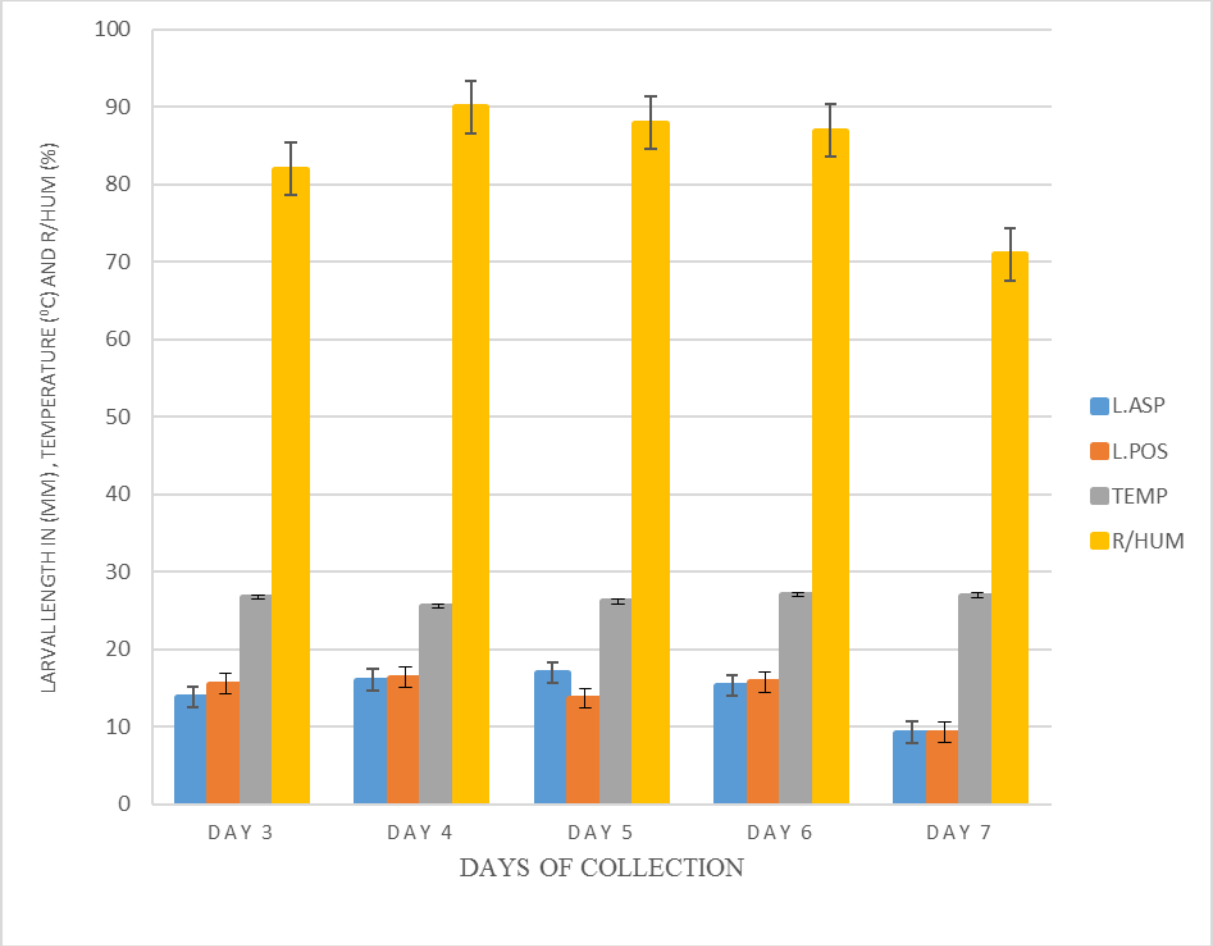


Figure 4.13: Mean length (in mm) of calliphorid larvae recovered from the poisoned and asphyxiated cadavers at the forest with their temperature and relative humidity during the rainy season in Awka, Nigeria

Note: L.ASP & L.POS= larvae recovered on asphyxiated and poisoned cadavers, TEMP & R/HUM= temperature and relative humidity

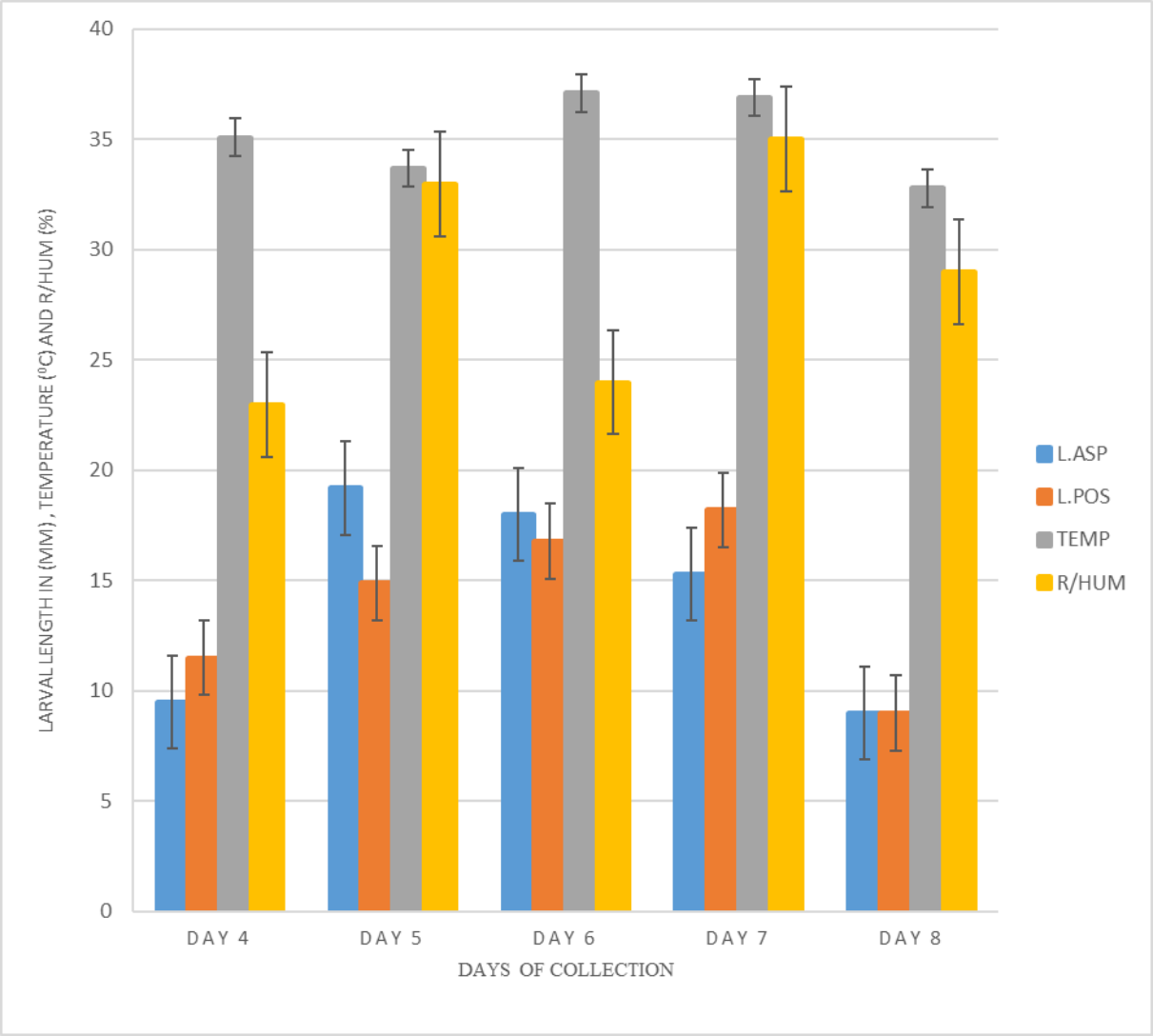


Figure 4.14: Mean length (in mm) of calliphorid larvae recovered from the poisoned and asphyxiated cadavers in the building with their temperature and relative humidity during the dry season in Awka, Nigeria

Note: L.ASP & L.POS= larvae recovered on asphyxiated and poisoned cadavers, TEMP & R/HUM= temperature and relative humidity

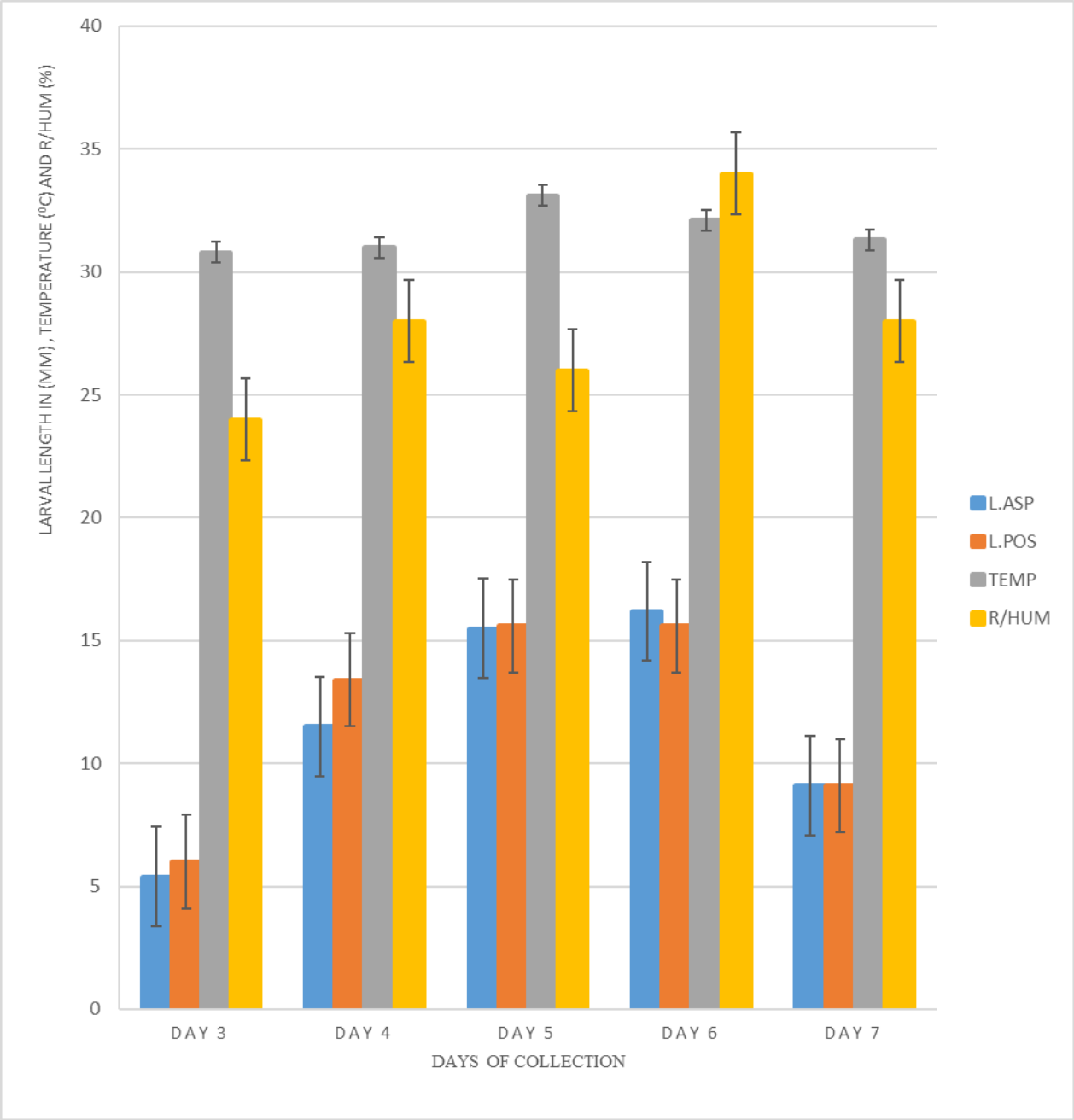


Figure 4.15: Mean length (in mm) of calliphorid larvae recovered from the poisoned and asphyxiated cadavers at the forest with their temperature and relative humidity during the dry season in Awka, Nigeria

Note: L.ASP & L.POS= larvae recovered on asphyxiated and poisoned cadavers, TEMP & R/HUM= temperature and relative humidity

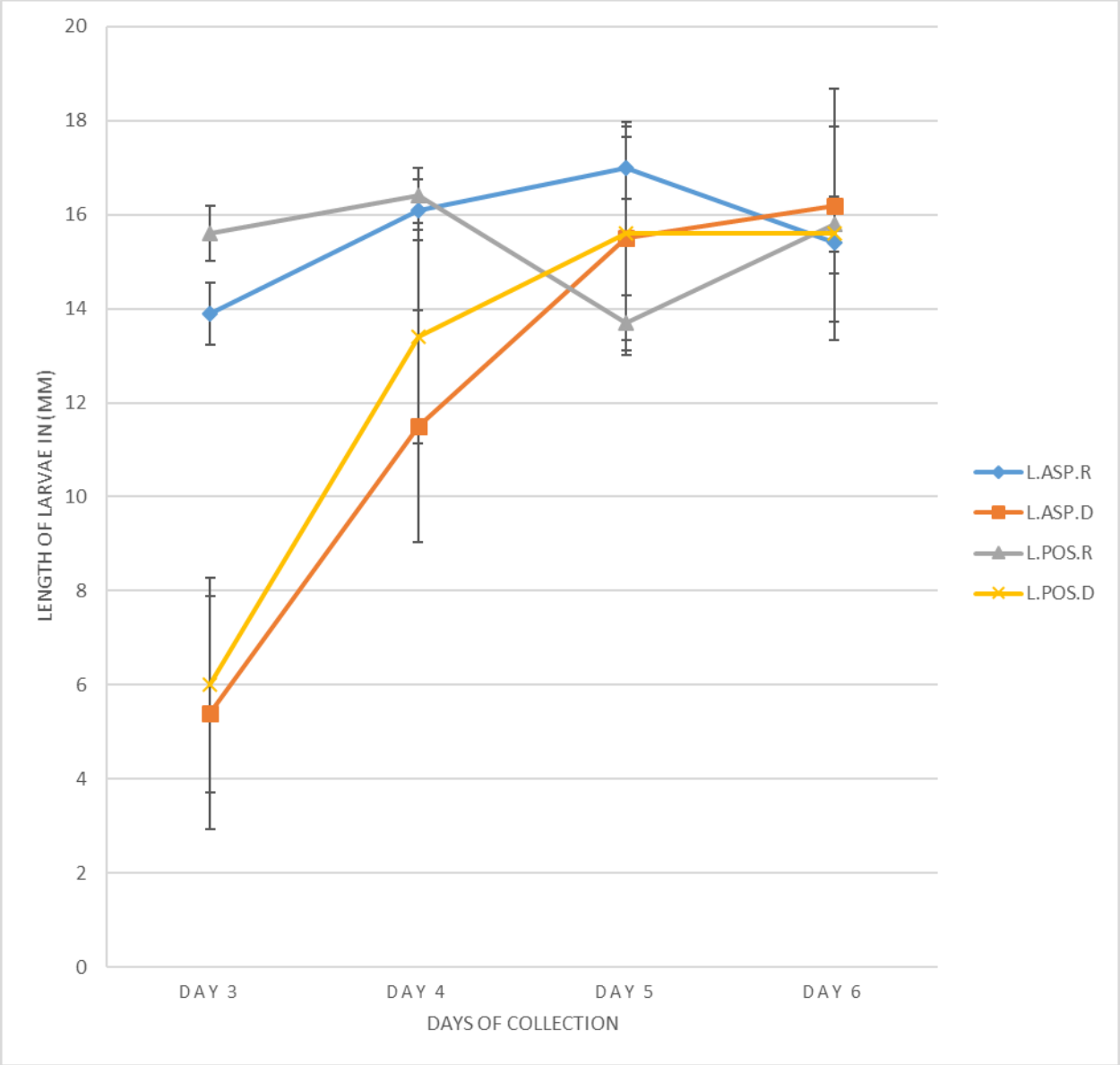


Figure 4.16: Mean length (in mm) of calliphorid larvae recovered from the poisoned and asphyxiated cadavers at the forest during rainy and dry season in Awka, Nigeria.

Note: L.ASP.R, L.ASP.D& L.POS.R, L.POS.D = larvae recovered on asphyxiated and poisoned cadavers during rainy and dry season

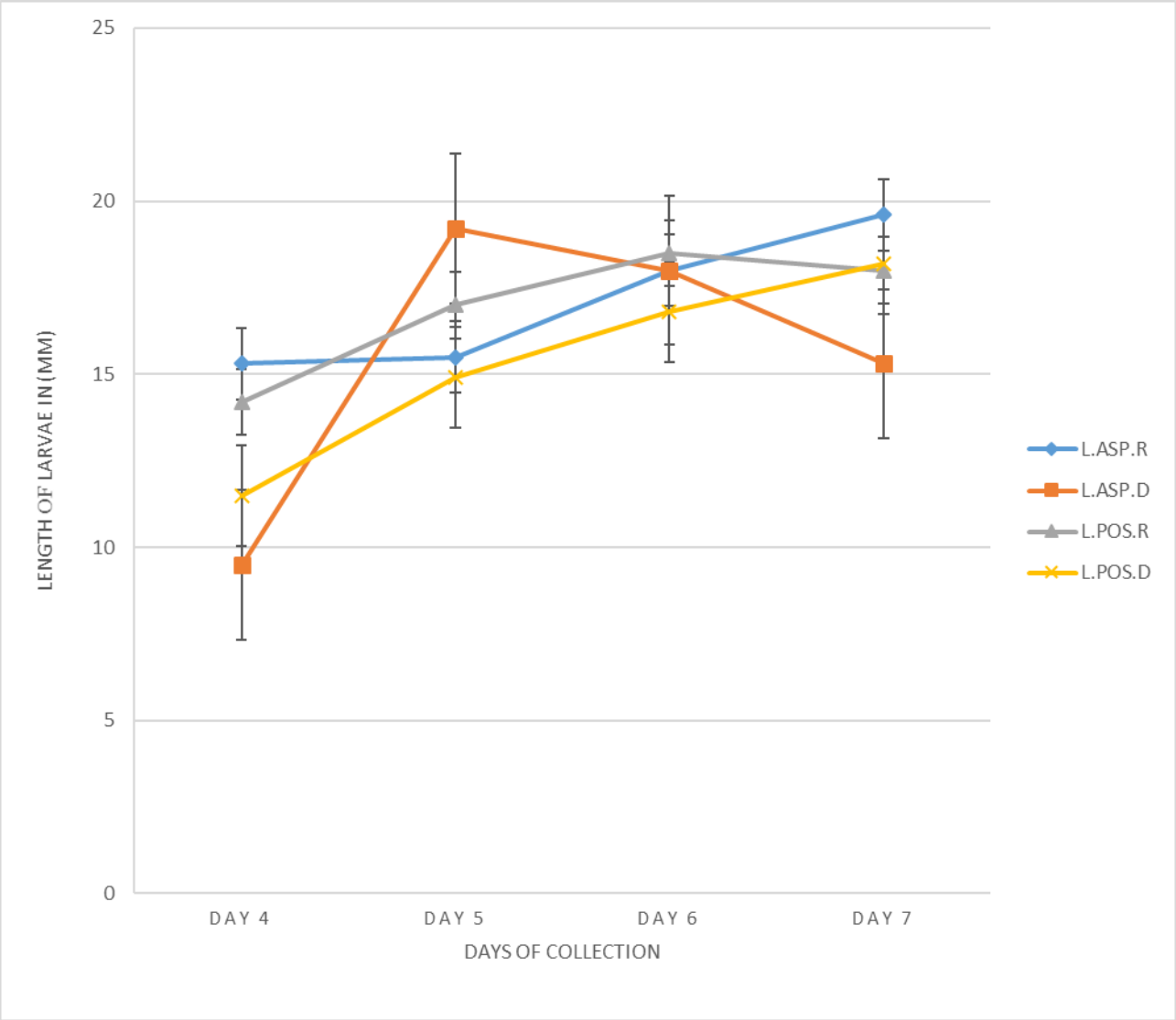


Figure 4.17: Mean length (in mm) of calliphorid larvae recovered from the poisoned and asphyxiated cadavers in the building during rainy and dry season in Awka, Nigeria

Note: L.ASP.R, L.ASP.D& L.POS.R, L.POS.D = larvae recovered on asphyxiated and poisoned cadavers during rainy and dry season

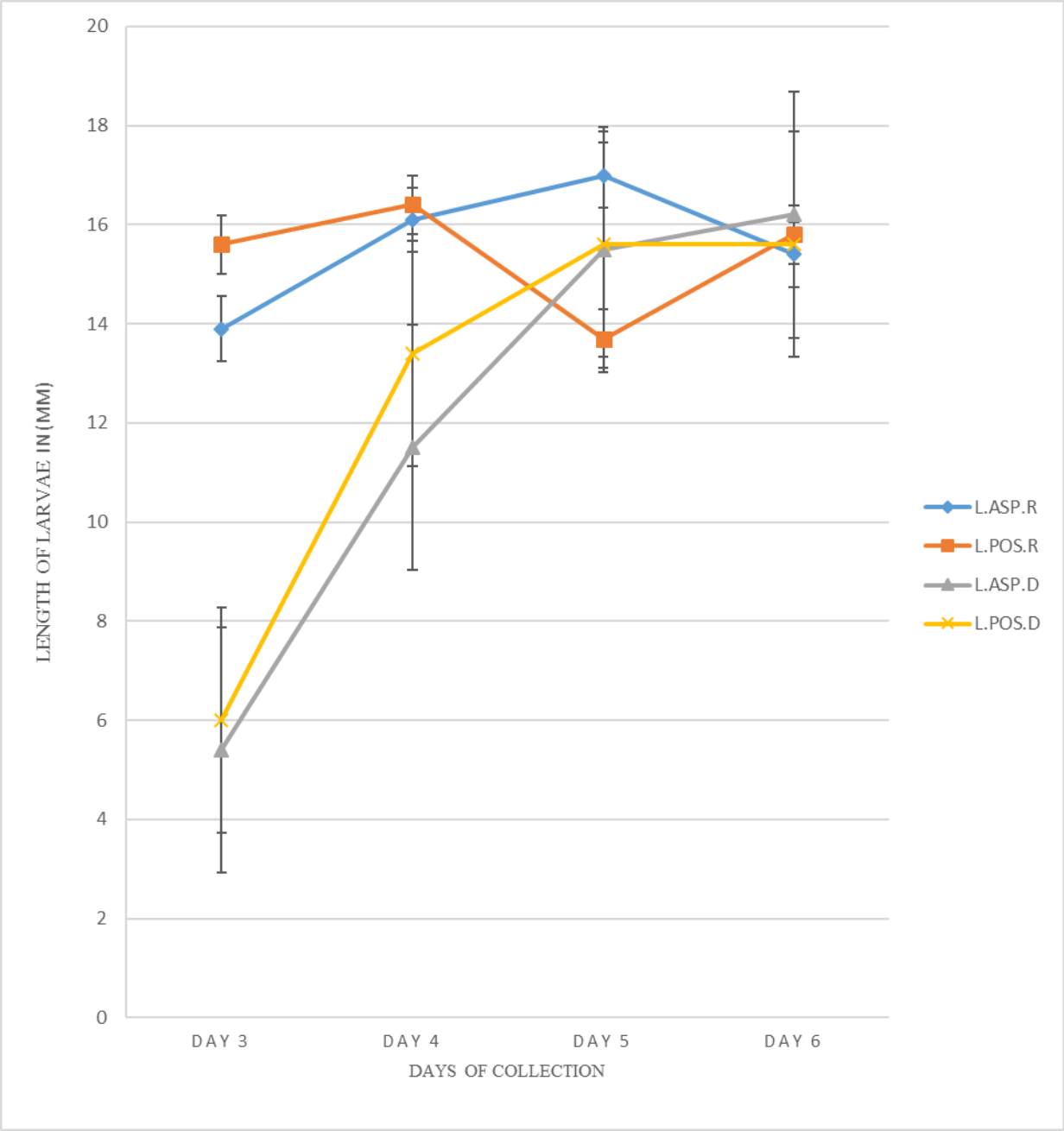


Figure 4.18: Mean length (in mm) of calliphorid larvae recovered from the poisoned against asphyxiated cadavers at the forest during rainy and dry season in Awka, Nigeria

Note: L.ASP.R, L.ASP.D& L.POS.R, L.POS.D = larvae recovered on asphyxiated and poisoned cadavers during rainy and dry season

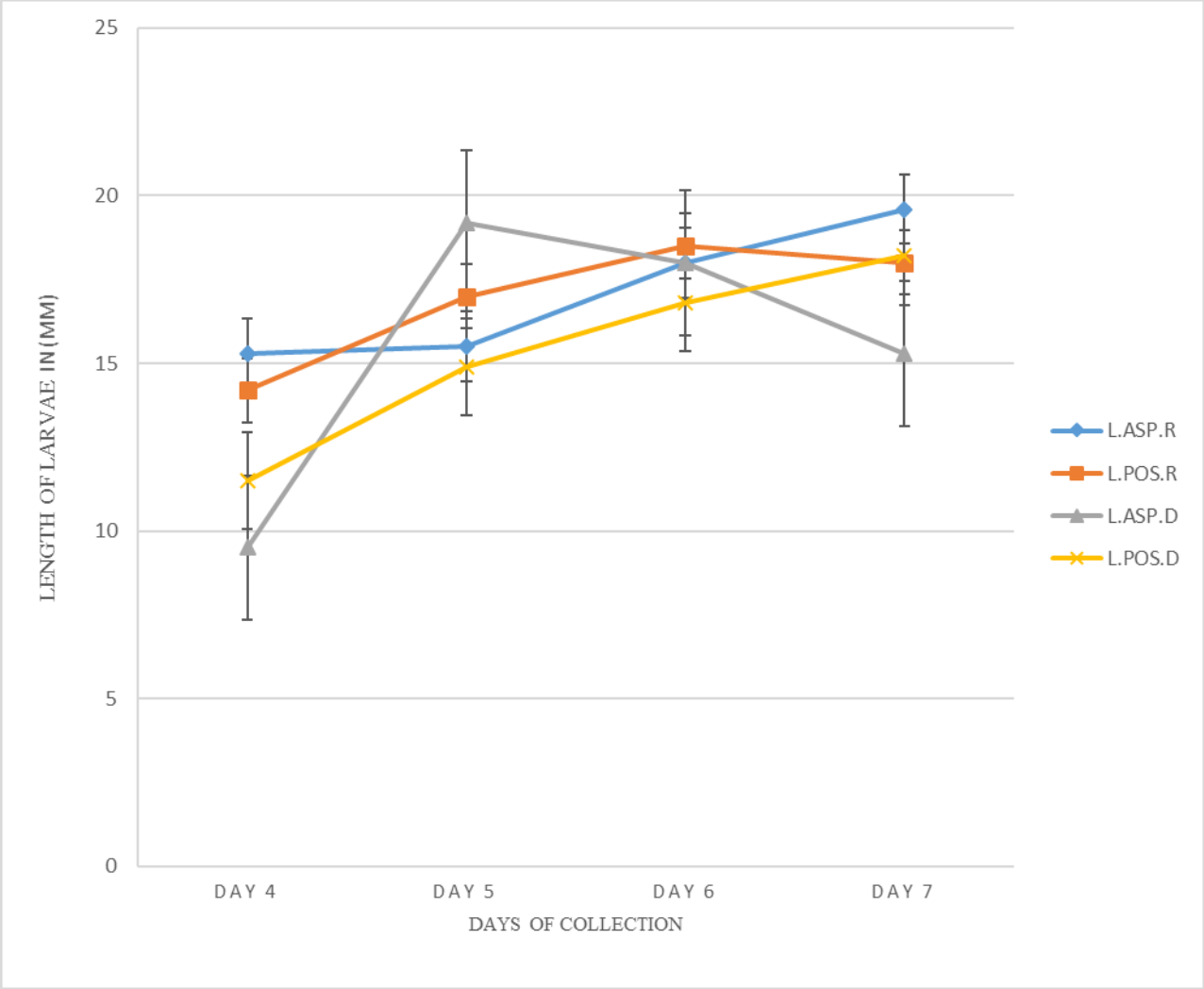


Figure 4.19: Mean length (in mm) of calliphorid larvae recovered from the poisoned against asphyxiated cadavers in the building during rainy and dry season in Awka, Nigeria

Note: L.ASP.R, L.ASP.D & L.POS.R, L.POS.D = larvae recovered on asphyxiated and poisoned cadavers during rainy and dry season

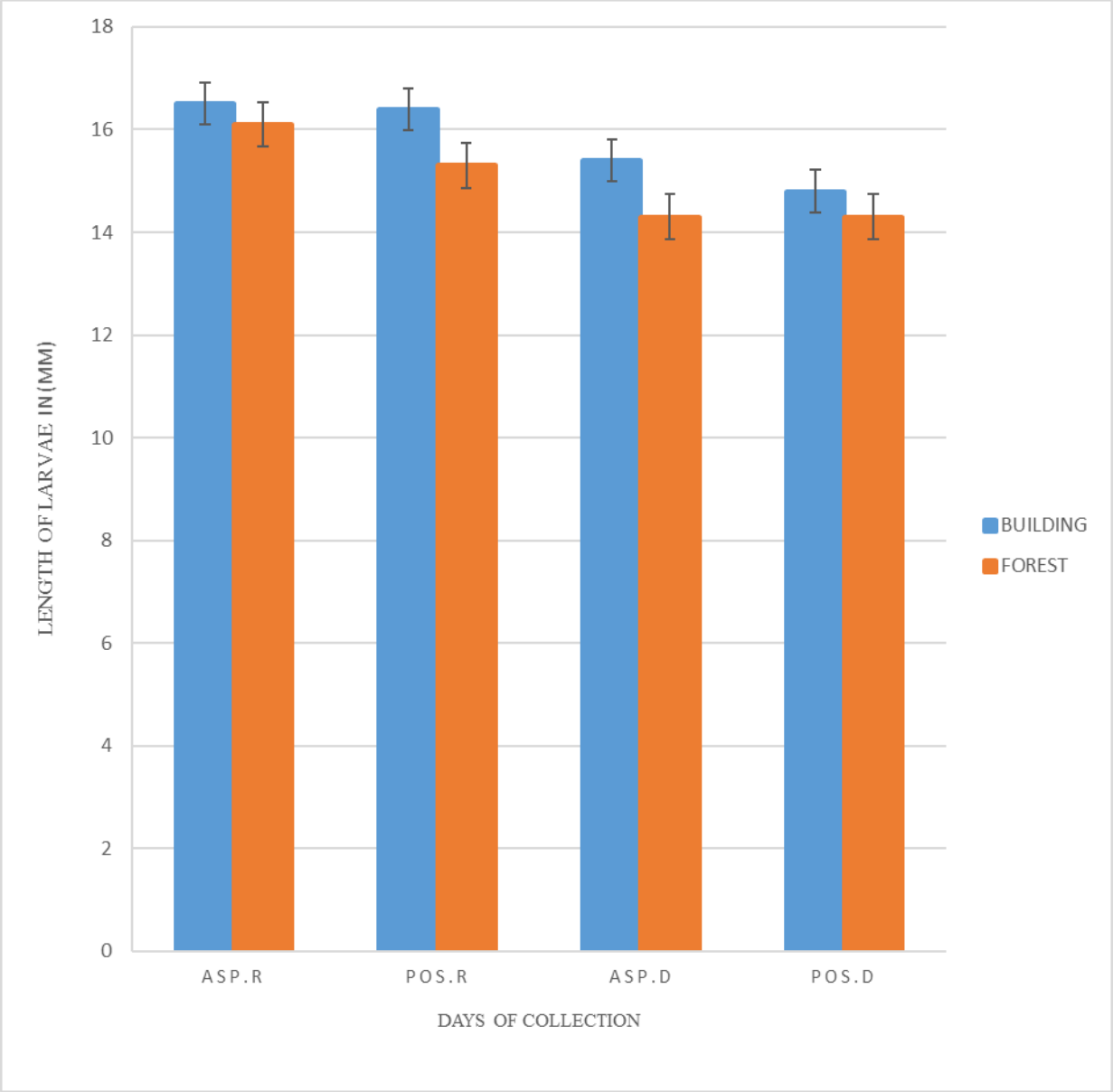


Figure 4.20: Mean length (in mm) of calliphorid larvae recovered from the poisoned and asphyxiated cadavers at the forest against the building during the rainy and dry season in Awka, Nigeria

Note: ASP.R, ASP.D & POS.R, POS.D = larvae recovered on asphyxiated and poisoned cadavers during rainy and dry season

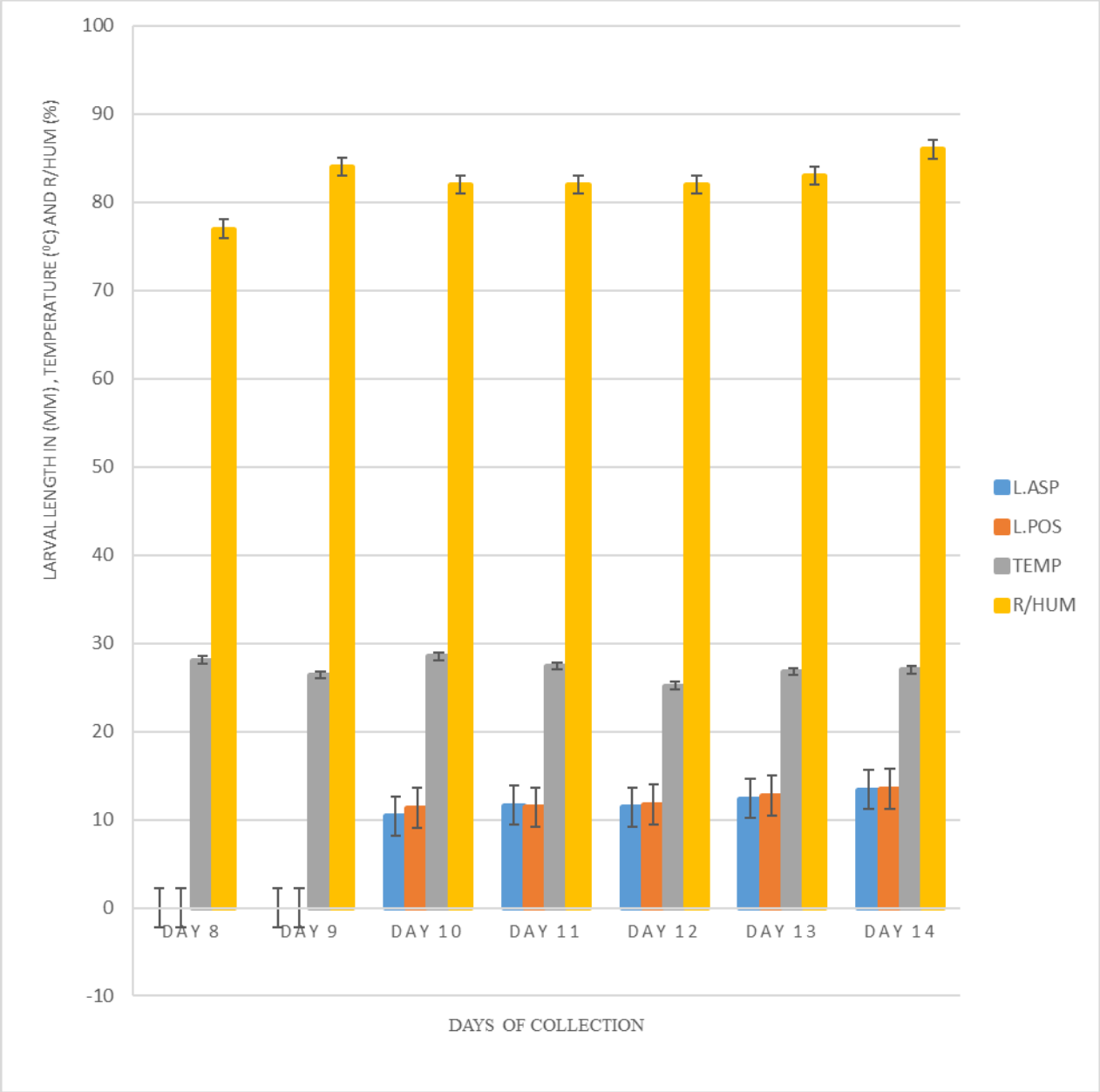


Figure 4.21: Mean length (in mm) of ulidiid larvae recovered from the poisoned and asphyxiated cadavers in the building with their temperature and relative humidity during the rainy season in Awka, Nigeria

Note: L.ASP & L.POS= larvae recovered on asphyxiated and poisoned cadavers, TEMP & R/HUM= temperature and relative humidity

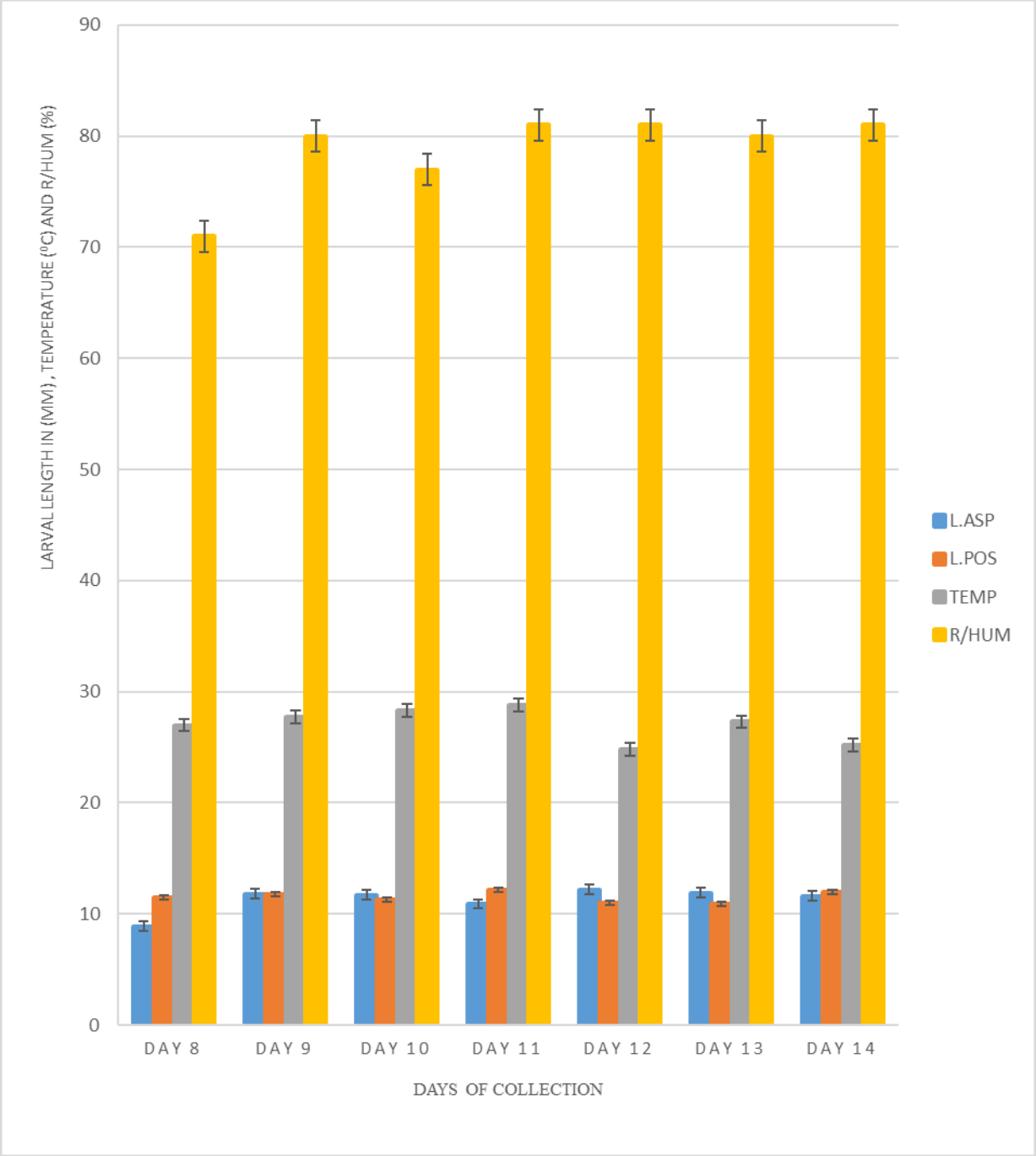


Figure 4.22: Mean length (in mm) of ulidiid larvae recovered from the poisoned and asphyxiated cadavers at the forest with their temperature and relative humidity during the rainy season in Awka, Nigeria

Note: L.ASP & L.POS= larvae recovered on asphyxiated and poisoned cadavers, TEMP & R/HUM= temperature and relative humidity

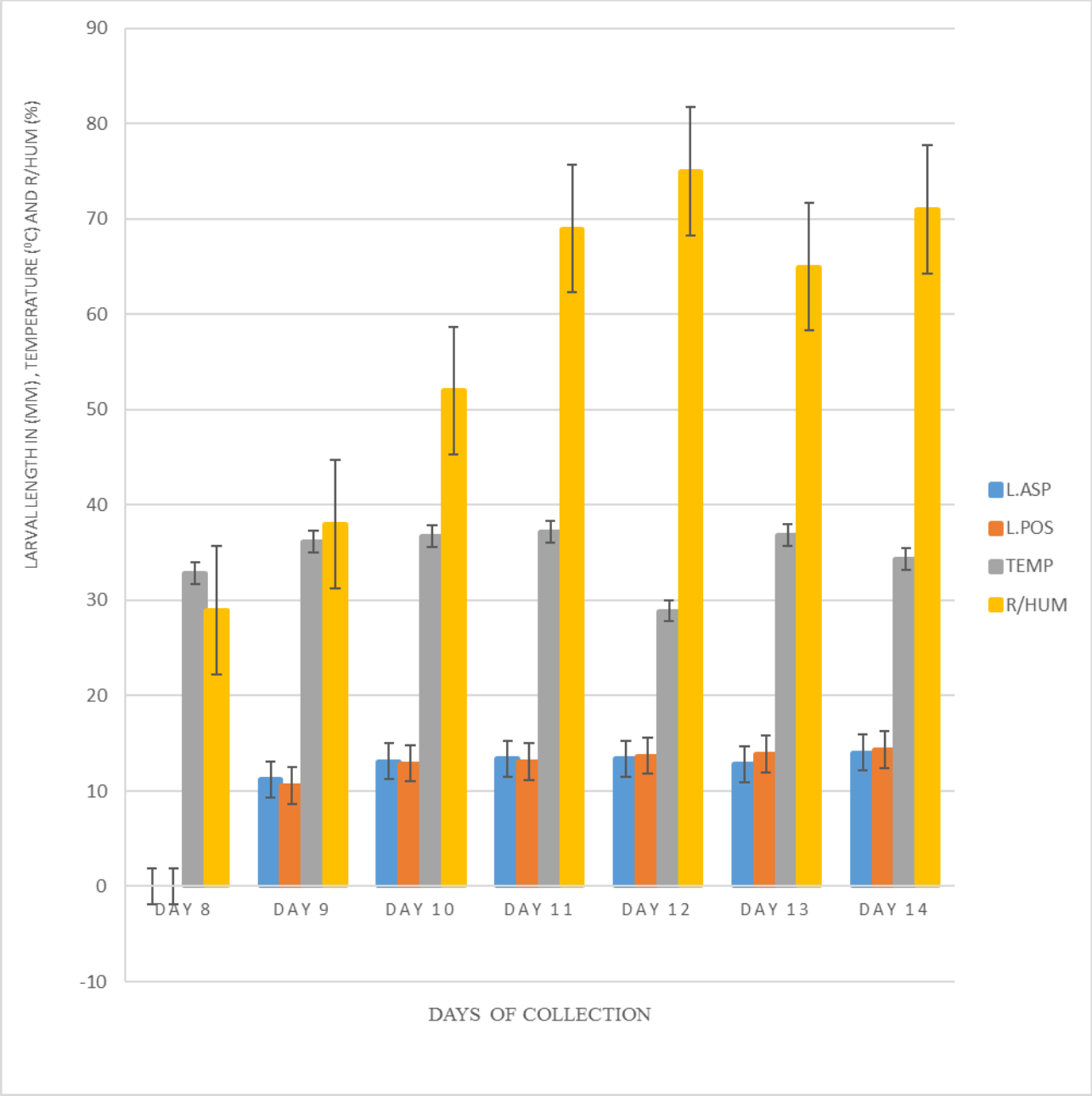


Figure 4.23: Mean length (in mm) of ulidiid larvae recovered from the poisoned and asphyxiated cadavers in the building with their temperature and relative humidity during the dry season in Awka, Nigeria

Note: L.ASP & L.POS= larvae recovered on asphyxiated and poisoned cadavers, TEMP & R/HUM= temperature and relative humidity

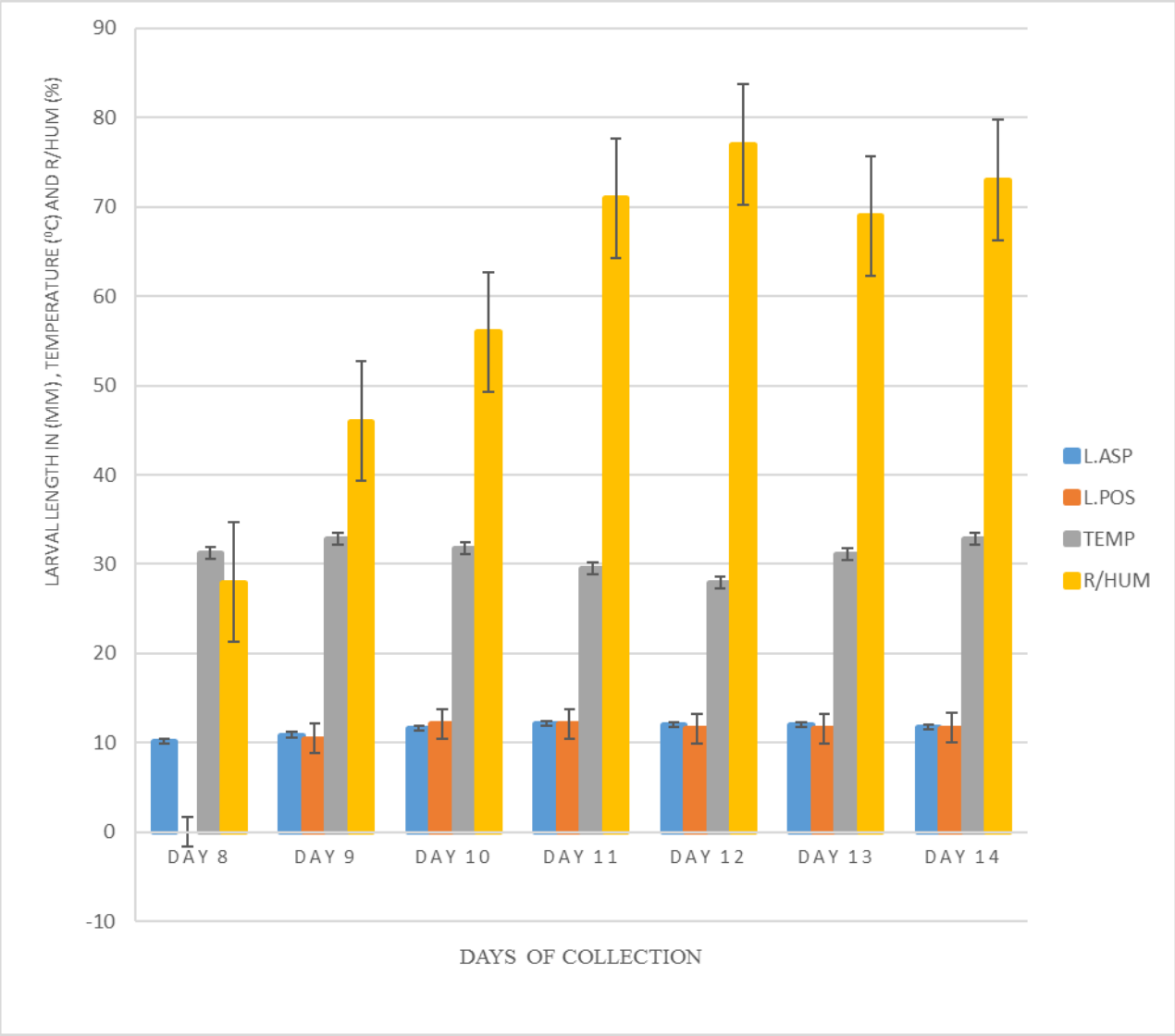


Figure 4.24: Mean length (in mm) of ulidiid larvae recovered from the poisoned and asphyxiated cadavers at the forest with their temperature and relative humidity during the dry season in Awka, Nigeria

Note: L.ASP & L.POS= larvae recovered on asphyxiated and poisoned cadavers, TEMP & R/HUM= temperature and relative humidity

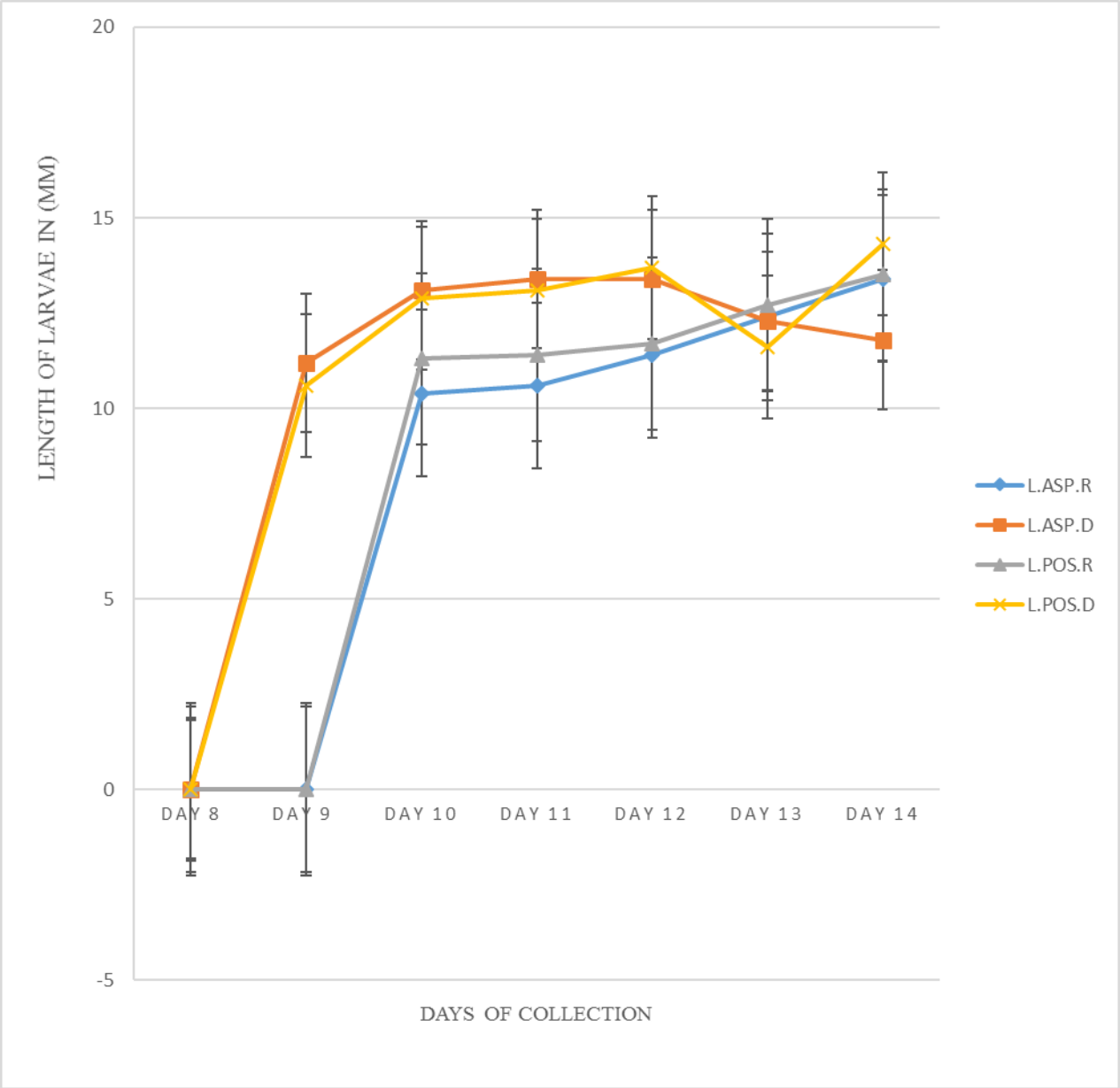


Figure 4.25: Mean length (in mm) of ulidiid larvae recovered from the poisoned and asphyxiated cadavers in the building during rainy and dry season in Awka, Nigeria

Note: L.ASP.R, L.ASP.D & L.POS.R, L.POS.D = larvae recovered on asphyxiated and poisoned cadavers during rainy and dry season

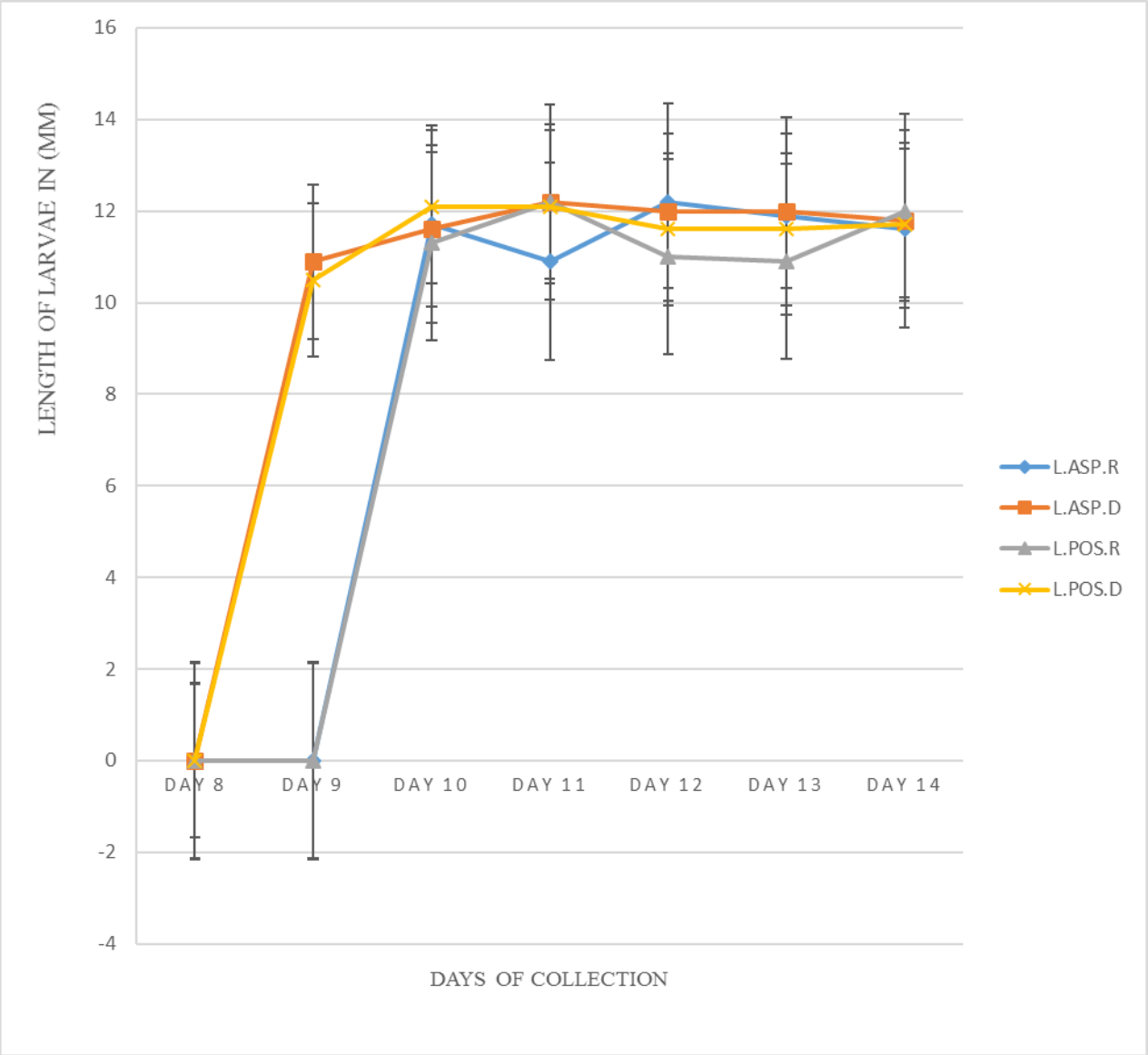


Figure 4.26: Mean length (in mm) of ulidiid larvae recovered from the poisoned and asphyxiated cadavers at the forest during rainy and dry season in Awka, Nigeria

Note: L.ASP.R, L.ASP.D & L.POS.R, L.POS.D = larvae recovered on asphyxiated and poisoned cadavers during rainy and dry season

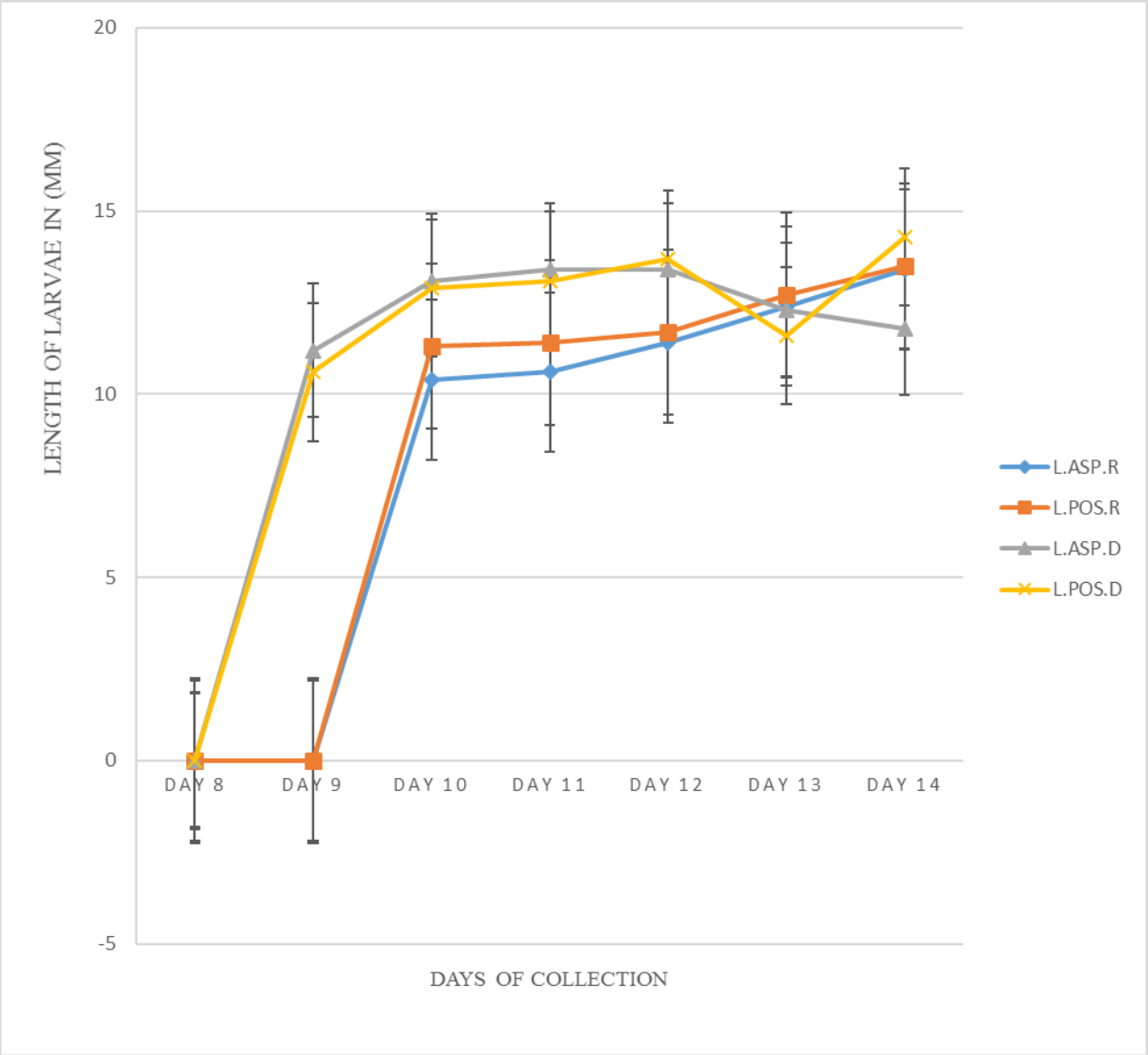


Figure 4.27: Mean length (in mm) of ulidiid larvae recovered from the poisoned against asphyxiated cadavers in the building during rainy and dry season in Awka, Nigeria

Note: L.ASP.R, L.ASP.D & L.POS.R, L.POS.D = larvae recovered on asphyxiated and poisoned cadavers during rainy and dry season

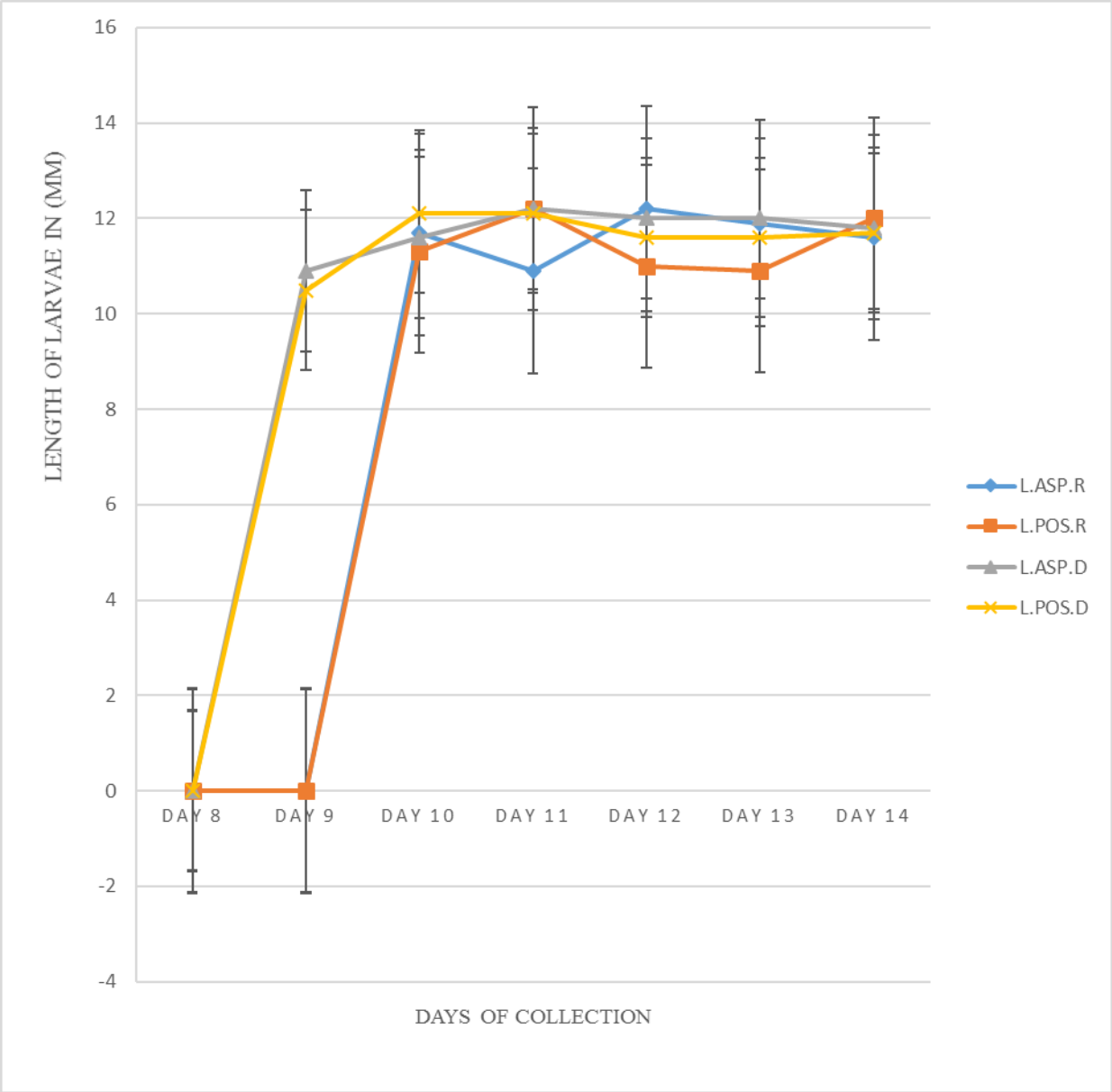


Figure 4.28: Mean length (in mm) of ulidiid larvae recovered from the poisoned against asphyxiated cadavers at the forest during rainy and dry season in Awka, Nigeria

Note: L.ASP.R, L.ASP.D & L.POS.R, L.POS.D = larvae recovered on asphyxiated and poisoned cadavers during rainy and dry season

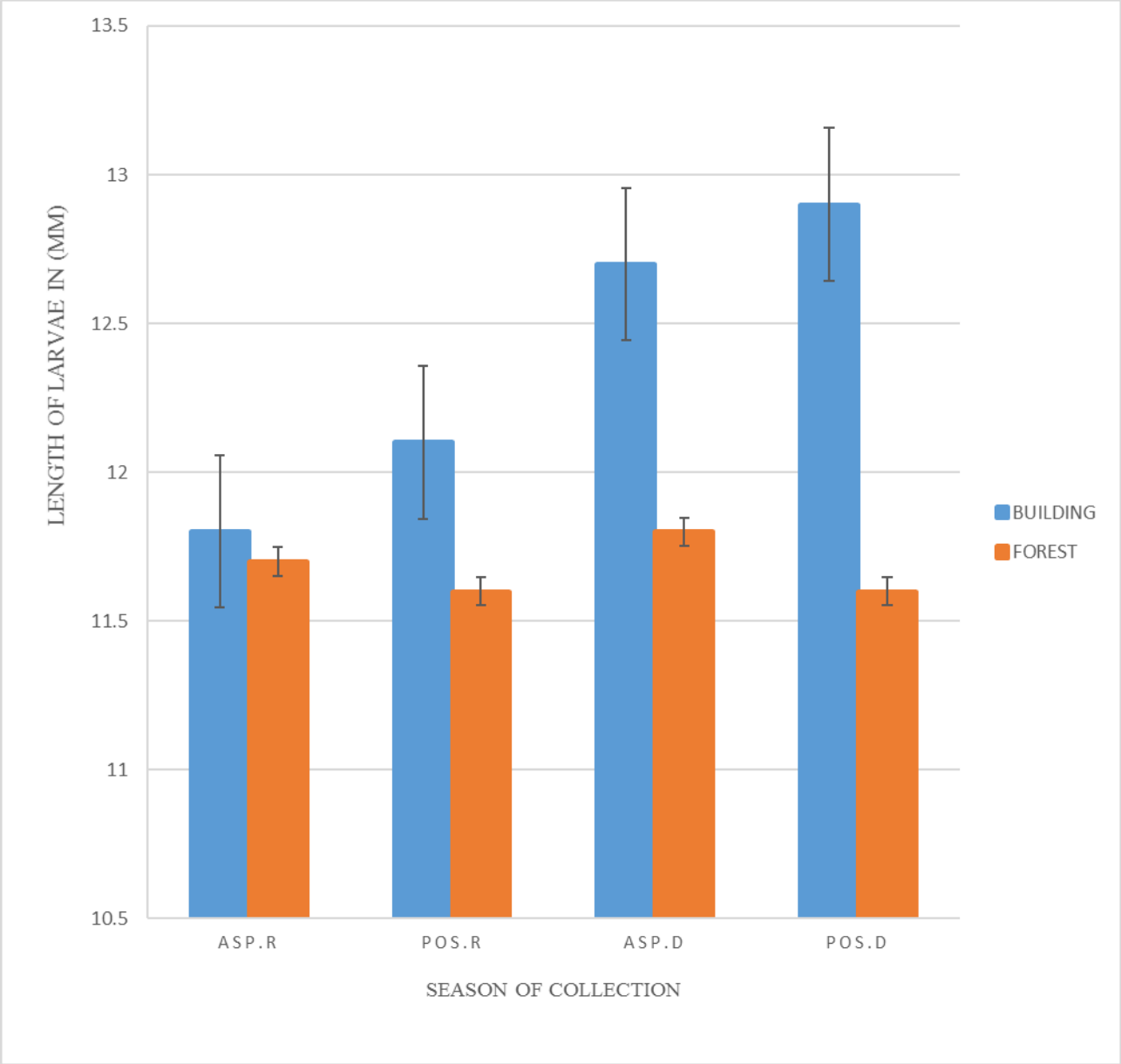


Figure 4.29: Mean length (in mm) of calliphorid larvae recovered from the cadavers in the building against forest during rainy and dry season in Awka, Nigeria

Note: ASP.R, ASP.D & POS.R, POS.D = larvae recovered on asphyxiated and poisoned cadavers during rainy and dry season

.

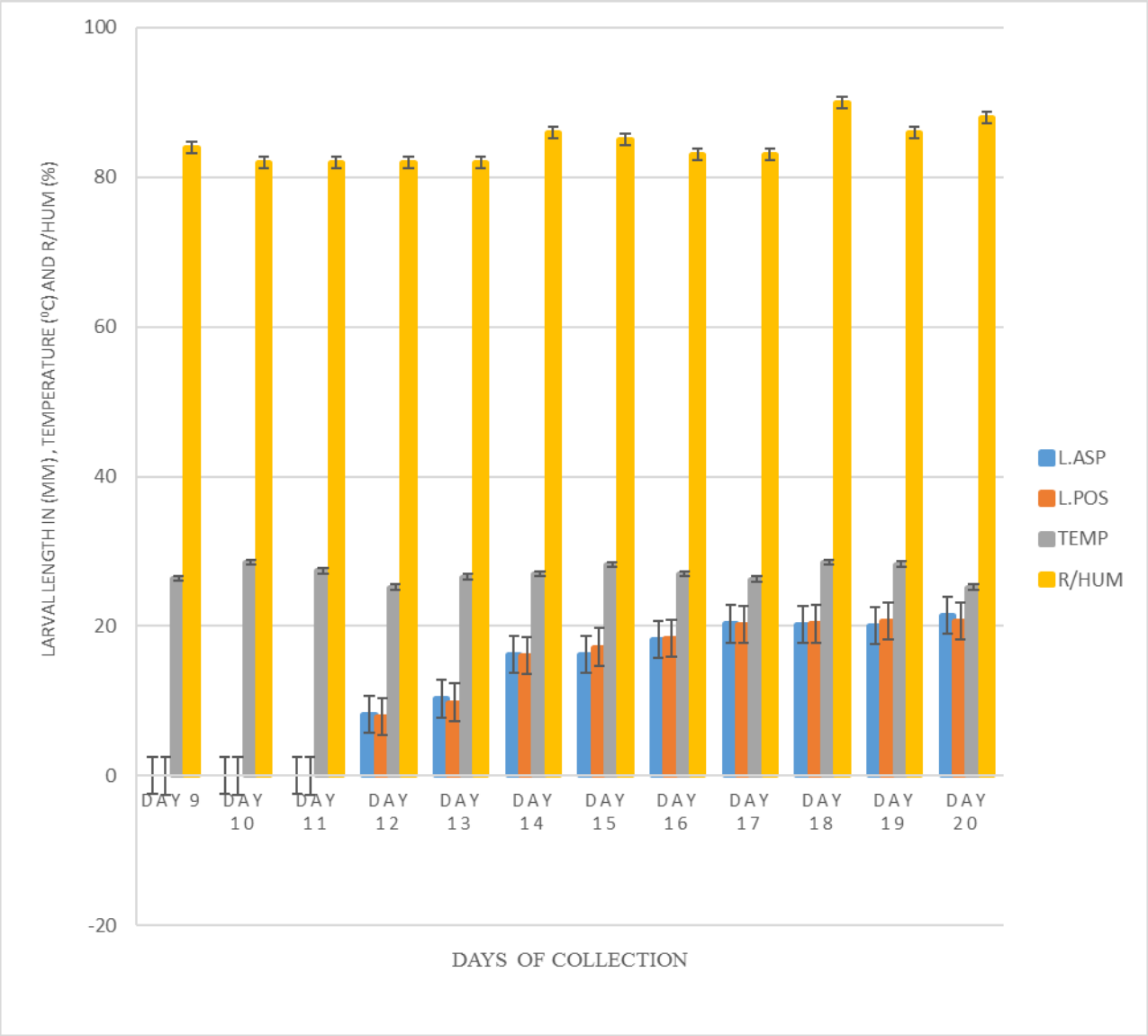


Figure 4.30: Mean length (in mm) of stratiomyiid larvae recovered from the poisoned and asphyxiated cadavers in the building with their temperature and relative humidity during the rainy season in Awka

Note: L.ASP & L.POS= larvae recovered on asphyxiated and poisoned cadavers, TEMP & R/HUM= temperature and relative humidity

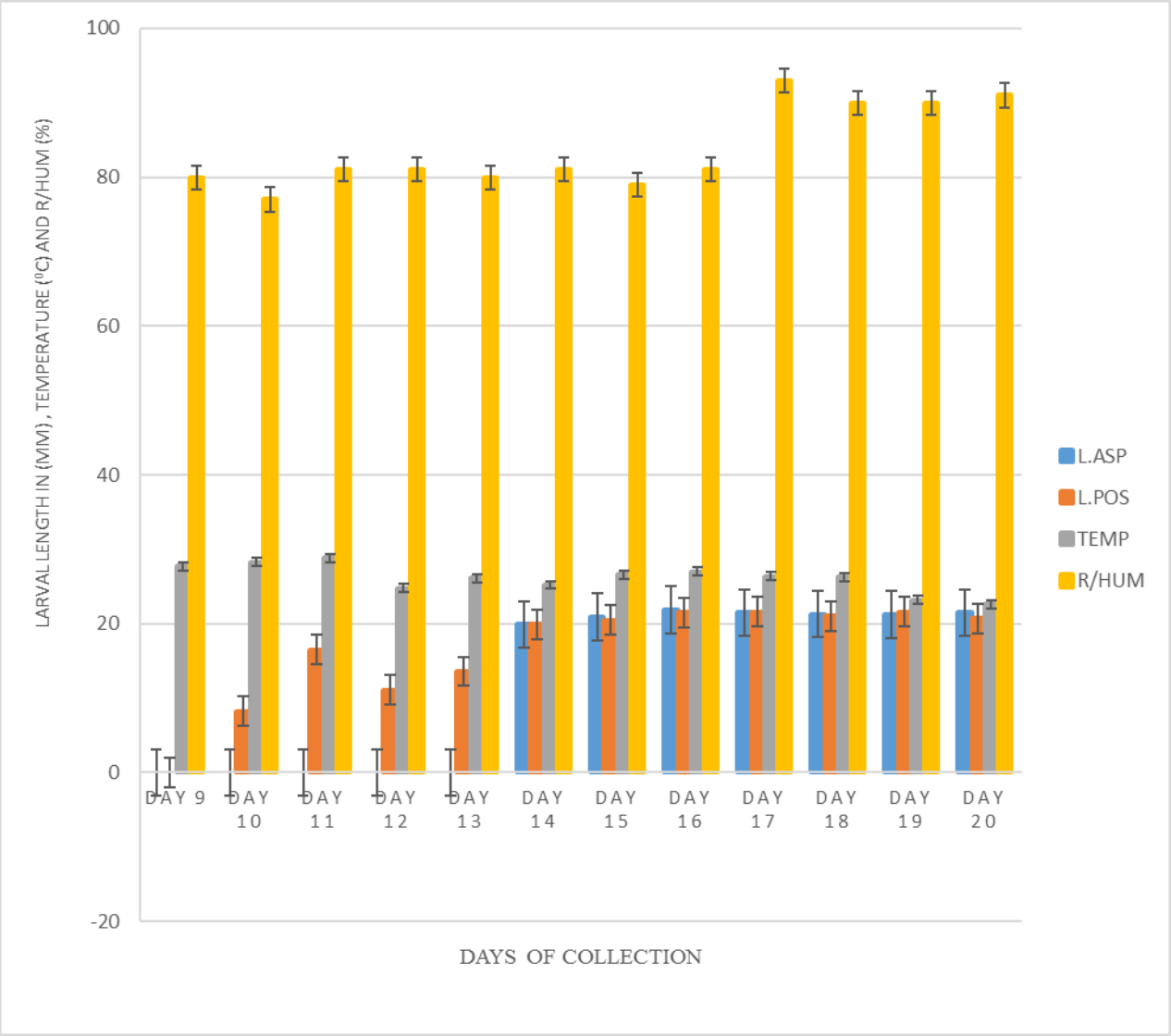


Figure 4.31: Mean length (in mm) of stratiomyiid larvae recovered from the poisoned and asphyxiated cadavers at the forest with their temperature and relative humidity during the rainy season in Awka, Nigeria

Note: L.ASP & L.POS= larvae recovered on asphyxiated and poisoned cadavers, TEMP & R/HUM= temperature and relative humidity

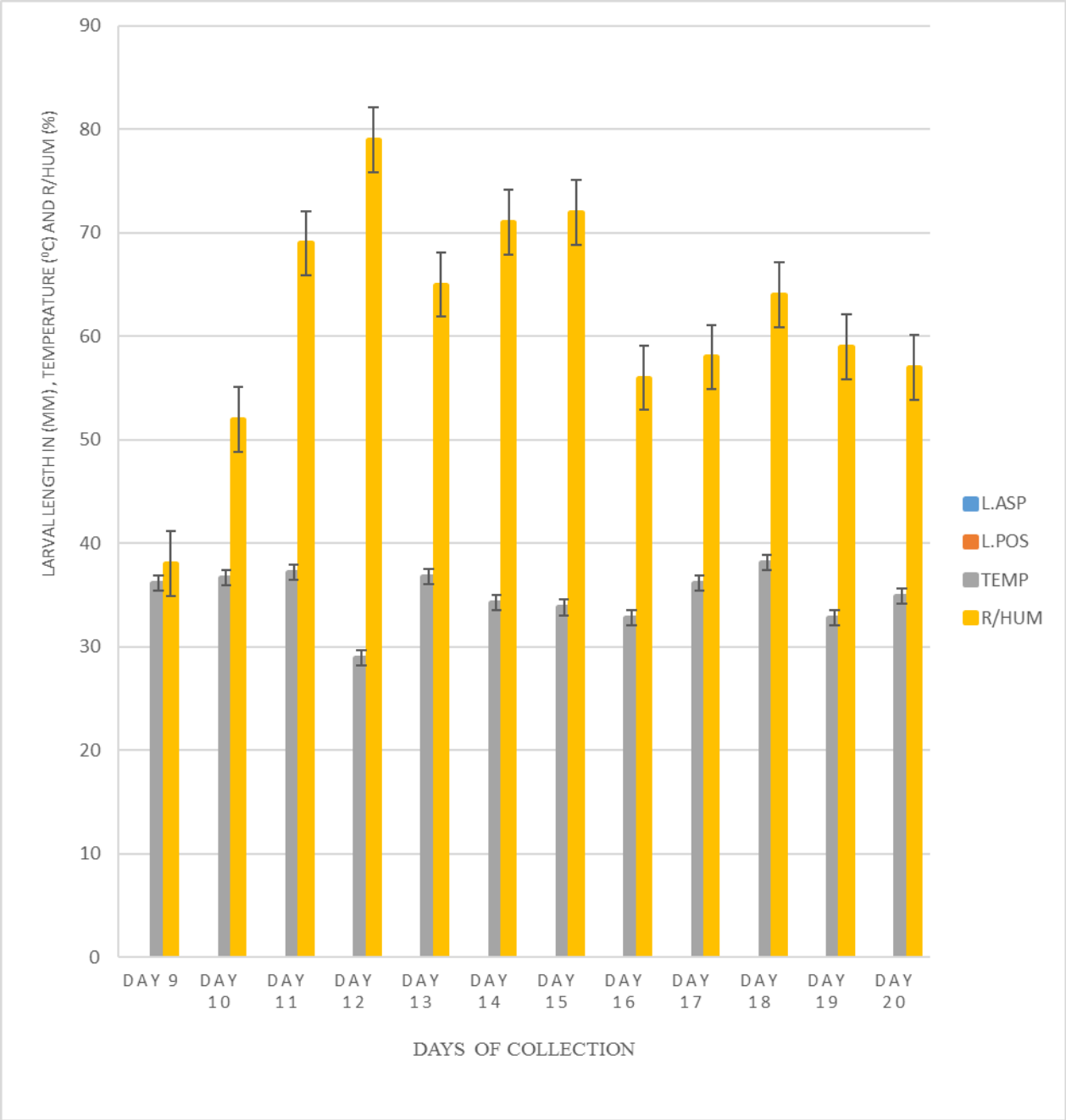


Figure 4.32: Mean length (in mm) of stratiomyiid larvae recovered from the poisoned and asphyxiated cadavers in the building with their temperature and relative humidity during the dry season in Awka, Nigeria

Note: L.ASP & L.POS= larvae recovered on asphyxiated and poisoned cadavers, TEMP & R/HUM= temperature and relative humidity

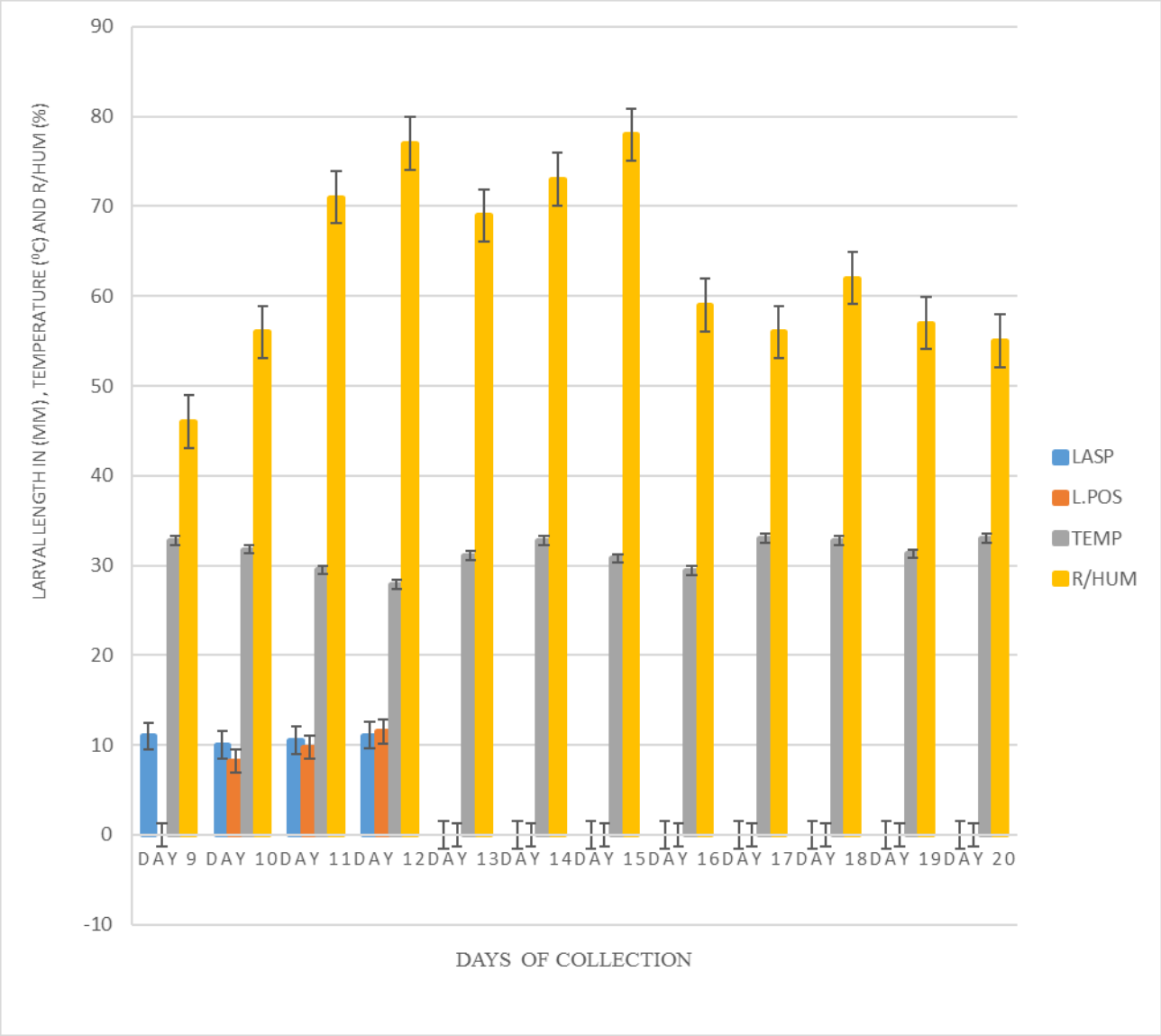


Figure 4.33: Mean length (in mm) of stratiomyiid larvae recovered from the poisoned and asphyxiated cadavers at the forest with their temperature and relative humidity during the dry season in Awka, Nigeria

Note: L.ASP & L.POS= larvae recovered on asphyxiated and poisoned cadavers, TEMP & R/HUM= temperature and relative humidity

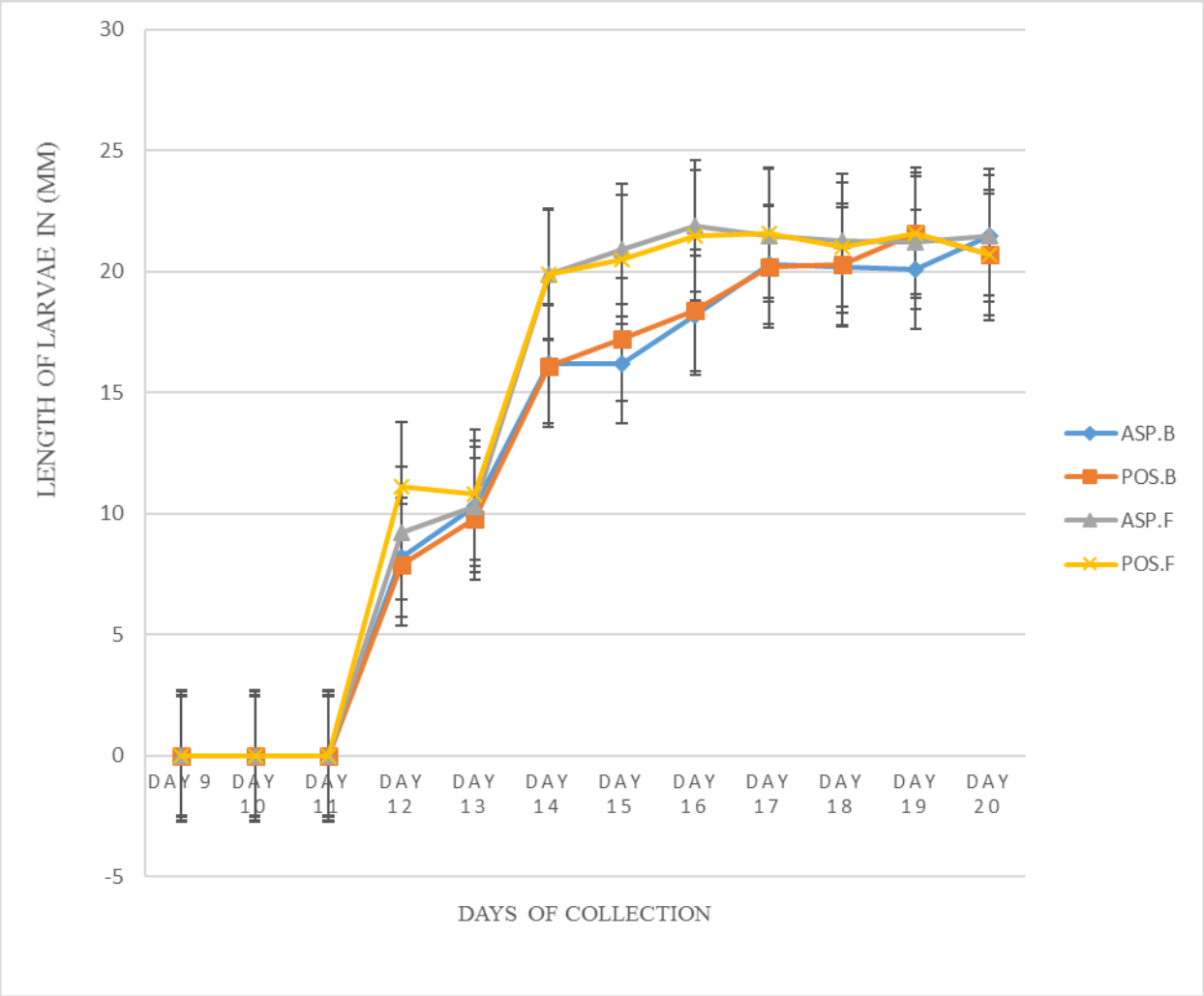


Figure 4.34: Mean length (in mm) of stratiomyiid larvae recovered from the poisoned and asphyxiated cadavers in the building and at the forest during rainy season in Awka, Nigeria

Note: ASP.B, POS.B & ASP.F, POS.F = larvae recovered on asphyxiated and poisoned cadavers in the building and at the forest

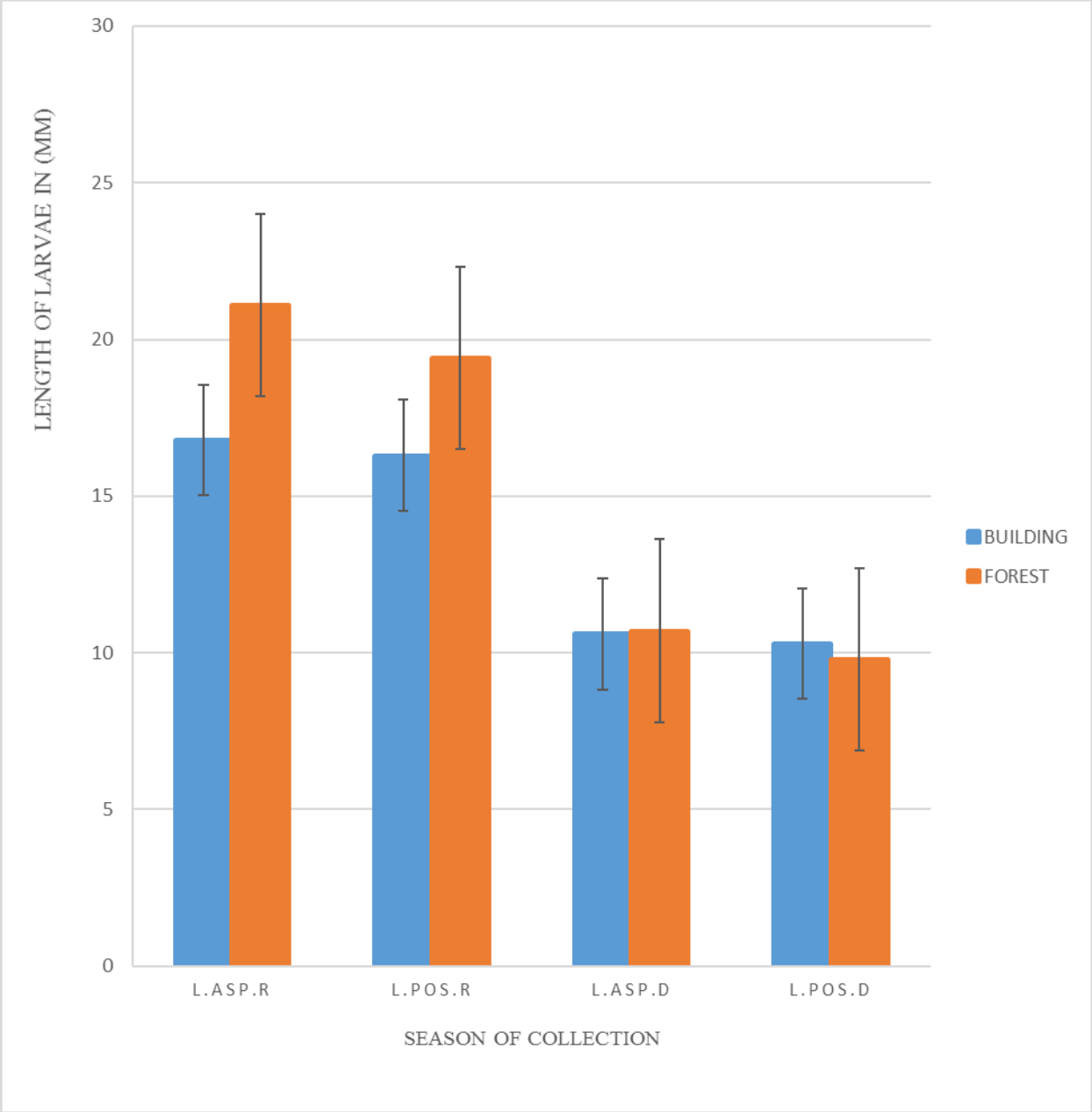


Figure 4.35: Mean length (in mm) of stratiomyiid larvae recovered from the poisoned and asphyxiated cadavers in the building and at the forest during rainy season in Awka, Nigeria

Note: ASP.R, ASP.D & POS.R, POS.D = larvae recovered on asphyxiated and poisoned cadavers during rainy and dry season

4.3.1. Assessment of zinc and phosphorus (the poison components of the rodenticide) on the cadaveric insect larvae and pupae

Toxicological analysis was performed on the larvae and pupae of calliphorid flies as well as on the larvae of ulidiid and stratiomyid flies with Atomic Absorption Spectrophotometer (AAS) to assess the zinc and phosphorus which were the active ingredients of the rodenticide. The AAS detected and quantified zinc but did not detect phosphorus (fig 4.38).

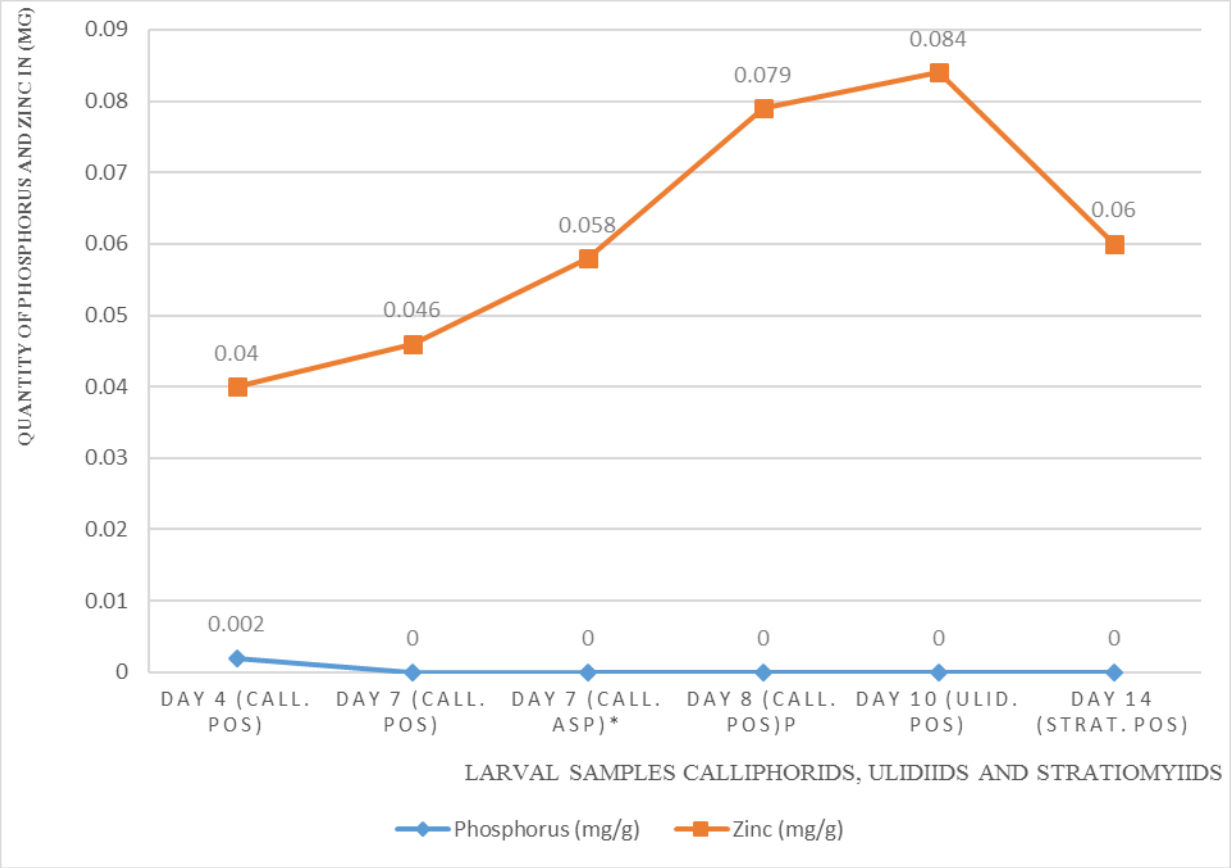


Figure 4.36: Quantity of phosphorus and zinc in (mg) detected from 1 g of the larvae and pupae samples

Note; day 4 and 7 (poisoned) were calliphorid larvae recovered on day 4 and 7, day 8 (poisoned) were calliphorid pupae recovered on day 8, day 10 and 14 were larvae of ulidiid and stratiomyid flies' recovered on day 10 and 14 respectively, Day 7 (asphyxiated) was calliphorid larvae recovered on day 7 as control.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Decomposition Stages of the Pig Cadavers and Their Associating Insects

Decomposing cadaver is organic matter every person would prefer avoidance approach. This is because of its unpleasant outlook and foul odour. It may be a source of various pathogens to the environment and its accompanied health implications. Ecologically, decomposing cadavers provide unique opportunity for scientists to investigate the cycle of organic nutrients in the ecosystem. It enhances the abilities to identify the factors influencing decomposition rates of cadavers and unravel the mysteries surrounding the unexplained deaths of animals, including humans (Mondor *et al*., 2012). Decomposition process of dead animals is described in terms of several successive phases or stages. This succession is however, micro-successive in nature with possible distinction into three to eight stages. The most important aspect of these stages is that every stage is predictable with its corresponding entomofauna.

In this study, four and five decomposition stages were identified during the rainy reason and dry season respectively. However, recognition of these various stages is a debate with divergent views. Thus, Bornemissza (1956) and Payne (1965) recognized five stages. Reed, (1958) recognized four stages while Fuller (1934) recognized three stages. The recognition of four and five stages of decomposition in this study is thus agreeable with recent studies which recognized a stretch of five stages: fresh, bloated, active decay, advanced decay and remains (Tantawi *et al*., 1996; Byrd and Castner, 2010; Goff, 2010; Ekrakene, 2012; Ndueze *et al*., 2013a).

The four stages distinguished in the decomposition of the pig cadavers in this study during the rainy season were fresh, bloated, wet decay and remains decay. This is in line with the reports of (Reed, 1958; Tantawi *et al*., 1996). The absence of dry decay or advanced decay in this work is attributed to frequent rainfall recorded during the active decompositions of the cadavers which saturated the air molecules and maintained relative humidity at 82.3±0.81% and the air temperature at 27.4±0.69 °C. Thus, this condition made the period of the study wet while the frequent rainfall returned the would be dry decay stage back to wet decay. Hence, the recognition of the four stages at both locations of the study. However, the stages of decomposition recognized during the dry season are contradictory to rainy season because there were no rainfalls during the active decomposition stages of the cadavers. This condition caused dry air molecules and lowers the relative humidity to 42.5±0.64% and increased the air temperature to 32.5±0.35 °C. Thus, the decomposing pig cadavers successively underwent five stages of decay: fresh, bloated, wet decay, dry decay and remains decay in line with (Bornemissza, 1956; Payne, 1965) who observed five stages of decomposition.

It is obvious from this work that there were marked differences in the decomposition of the pig cadavers in the two seasons. In effect, the two contrasting environments were similarly affected during each season. This study attributed this finding to similar temperature and relative humidity recorded at the two environments hence, the same decomposition rates were observed at every season irrespective of the study locations. Apparently, the high moisture content of the rainy season made the environment humid thus excluding the dry decay stage while the low moisture content during the dry season made the air molecules dry thus, dehydrated the cadavers faster and as well delayed bacteria action and desiccated the

carcasses to depict a dry decay stage. This is the reason for the pig cadavers to decompose completely in 10 days during the rainy season and 14 days during the dry season despite using the same weight of pig cadavers throughout the study period.

Relatively, over 95% of specimens on cadavers are insects (Villet, 2011). The majority of the insects recovered from the decomposing pig cadavers belong to the orders: Diptera, Coleoptera and Hymenoptera. The dipterans are early colonizers dominated by the family, Calliphoridae while the coleopterans constitute the late and drier colonizers dominated by the families; Cleridae and Dermesitdae. However, the guild of hymenopterans especially the family Formicidae was similar in all the stages of decomposition and played roles that are ecologically recognized in contrast to the dipterans and the coleopterans which have been recognized as forensic insects.

Empirical studies relating to cadaver decomposition affirmed that insects may locate carcass few minutes after death (Gennard, 2007; Byrd and Castner, 2010) and their arrival is in succession in response to successive decomposition of the carcasses (Smith, 1986). In this study, insects recovered on the pig cadavers during the fresh stages of the cadavers during the rainy season include *Chrysomya albiceps, C. chloropyga, C. regalis* (calliphorids), *Pheidole* sp. and *Camponotus acvapimensis* (formicids). This observation conformed to the findings of Ekanem and Dike (2010). During the dry season, insects found were still the calliphorids and formicids in addition to *Sarcophaga inzi,* (sacophagids) and *Chrysomyza africana* (ulidiids). The difference in the species richness of the cadavers during the two seasons were attributed to extension of the fresh decay stage from day 1 to day 2 during the dry season due to dryness of the atmosphere which delayed bacteria action in the cadavers and hence, increased the

insect fauna with mainly hymenopteran group. Therefore, the abundance and the distribution of the cadaveric insects partially fluctuated between the two seasons while their dynamics were not affected. Thus, the reports of Christiansen (1964) as cited by Ewuim (1996) that soil moisture and rainfall were generally the strongest correlate with densities of arthropod (collembolan) populations, is in consonance with the findings of this study in relation to influence of rainfall on the cadaveric fauna of this region.

The bloated decay stage also recorded the calliphorids which dominated the cadaveric tissues as well as their predators especially the histerids. The clerids and dermestids were found during the wet decay stage as well as good number of the histerids that dominated the interface of the cadavers, where they predate on the calliphorid larvae. However, during the dry decay and the remains stages, when the cadavers where only calcified materials (bones and teeth) with dried tendons and ligaments, the cadavers were dominated by clerids and dermestids. The dipteran fauna of this stage were the larvae of *Hermetia illucens* (Stratiomyidae). Nazni *et al*. (2011) also made similar findings on decomposing monkey carcass in Malaysia.

Chrysomyza africana (Ulidiidae) larvae, which were consistently observed from the study during the dry and remains decaying stages, which affirmed the reports of Abajue *et al.* (2013) that the presence of ulidiid larvae during the dry decay of a body can offer forensic investigators on homicide the postmortem interval of badly decomposed remains.

Interestingly, Villet (2011) was of the opinion that a mechanistic link is often made between the stages of decay and the activities of specific insects as it is regularly claimed in the

forensic literature that each micro stage has its own community. Therefore, the match between the stages and the occurrence of particular insects is erratic and the succession progressive, rather than episodic.

Apparently, it was observed from the present study that succession is progressive and that insects were progressively found during different decomposition stages. The study therefore, opined that if thorough succession table is developed while understanding the ecological variables of such region that affect decomposition and insect community, estimation of time of death will be possible. Thus, this work is of the opinion that insect species richness found on cadavers, present a logical case that can estimate the age of death. This is true, because in as much the major insect fauna found at one stage may be found at different stages, the absence or presence of one or two of the insect species can estimate the PMI. For instance, it was observed from the study that calliphorid larvae were found from fresh to remains stages as well as their empty pupariae. Thus, the absence of callphorid larvae and the presence of their empty pupariae is an evidence of 20 days PMI estimates. In addition, the presence of 1st larval instar of ulidiids was an evidence of 8 days PMI estimate as the day is marked with clusters of non-active flying calliphorid flies which where the first generation of the first batch of larvae that emerged from the cadaver. This, usually occur during the peak of wet decay during the rainy season and onset of dry decay during the dry season. Thus, the succession tables developed in this study presented the insects recovered at every stage of the decomposing cadaver, at every stage of insects development as well as at different stages of their arrival which this work agreed was progressive and not episodic. Therefore, a look at the cadaver tells the decomposition stage of the cadaver considering the environmental variables

while the insect species richness serves as the biological clock that estimates the age of the cadaver.

5.1.1 Insects of forensic importance recovered from the pig cadavers

Insect fauna recovered from cadaver or corpse is used in forensic investigations primarily to develop an estimate of when that animal or the person died (Villet, 2011). Smith, (1986) called the period postmortem interval PMI while Byrd and Castner, (2010) referred to it as time of colonization TOC or period of insect activity PIA. The information needed to make such ecological estimate as proposed by Villet (2011), include the identity of the insects (community composition), their role in the community (guild structure) and how the colonization process progressed (community dynamics).

While we consider cadaver as a functional ecosystem, it will make epistemological sense, as the guild structure and the community dynamics would be useful to forensic entomology (Villet, 2011). The insect species recovered in this study are similar with other forensic insects in relevant literatures as three insect orders: Diptera, Coleoptera and Hymenoptera stand out in this affirmation. In relation to forensic entomology, the dipterans especially the calliphorids have gained global recognition as flies that dominate decomposing cadavers. The larvae and adults of calliphorid flies are significant in forensic entomology (Smith, 1986; Beneck, 2004; Amendt *et al*., 2004; Gennard, 2007; Byrd and Castner, 2010).

In this study, the first calliphorid fly arrived on the pig cadavers three minutes after, at the forest, and six hours after, in the building during the rainy season. Similar arrival time was also observed during the dry season. The observation is in tandem with the findings of

Ekrakene, (2012). Therefore, it is assumed from the study that the arrival of insects on the cadavers was not affected by the seasonal differences occasioned by temperature and relative humidity singly manipulated by rainfall. However, rainfall may affect oviposition by washing away freshly deposited eggs from the outdoor cadavers while nocturnal condition may prevent oviposition by at least 12 hours especially in the building. However, the reports of Ekrakene (2012) that monocrotophos poison injected on pig carrions at Benin, Nigeria delayed flies oviposition between 6 and 7 hours were not validated in this work. The difference however may be because of the components of the poisons. Thus, while the monocrotophos poison caused oviposition delay, the zinc phosphide used as food poison to kill the pigs in this study did not delay flies oviposition on the cadavers. It was observed that the zinc phosphide caused acute kidney and heart failure in the pigs thus, forcing blood to exude from their nostrils and the mouth, therein attracting calliphorid flies the same time as the asphyxiated pig cadavers. The little delay observed in the arrival of calliphorid flies in the building was as a result of the enclosed nature of the building thus, preventing the odour within the enclosed building for hours until the finely tuned sense of smell of the calliphorids later detected the odour through the small openings between the roof and the walls of the building and thus infest the cadavers simultaneously. The arrival of the calliphorids and their eggs that hatched into larvae on the cadavers are preponderant and dominated other insects in number for the first 15 days.

Contrarily, the arrival and the number of sarcophagid flies were poor in this work. Just very few were spotted in the building during the rainy season and non at the forest. However, they were found in both locations during the dry season and were closer to the cadavers at the

indoor location than at the outdoor. This observation contradicted the reports of Abajue *et al*. (2013 and 2014) who observed them as common flies associating with pig carrion decomposition in Okija, Anambra State. Their poor presence in this study is attributed to the synanthropy in the rural areas where animal and human defecation as well as poor pit latrine conditions are common. This makes them to adapt in the rural environment due to poor sanitary condition occasioned by animal and human defecations hence they were alien to the locations of this present study where sanitary condition is high with regards to animal and human defeacations.

Relative to their fecundity, the few spotted sarcophagid flies of this study, observed only during the fresh and bloating stages could not larviposit on the cadavers because every part of the cadaver has been dominated by calliphorid flies and their larvae, leaving no space for their reproductive cycle. This observation was validated by the larval samples of this study collected for rearing which failed to implicate any sarcophagid fly. This also contradicted the reports of Abajue *et al*. (2014) who implicated the reared larval samples collected from pig carrions during the bloating stages of decomposition.

Sequel to flies of this study, the muscid flies being synanthropic and cosmopolitan were found throughout the study period at both locations. However, their preponderance during the dry season in the building when the cadavers were undergoing wet decay was interesting to note as nuisant fly guilds. None of the eggs or the larvae samples reared failed to implicate them. Their preponderance during the wet decay is evidence that they associate themselves with moist filthy environments. They usually lay eggs in moist filthy environments and their larvae develop freely to adult in a short duration. However, the absence of their larvae in this

study may be attributed to predation by blowfly larvae. Coe (1978) reported that blowflies during dispersing stage do predate on late developing fly larvae. Smith (1986) however argued that it is not clear how the regularly visiting house flies breed on carrions. Hence, this study assumed that their eggs and larvae were predated upon either by the blowfly larvae or other predating beetles that were conspicuous at that stage hence, the absence of the adults among the reared samples.

It was also observed that stratiomyiid flies were very few. About 2 to 3 of the flies were spotted closer to the cadavers during the bloated stage at the forest during the rainy season. Their eggs were seen during the wet decay and their 1^{st} larval instars were seen between the 9^{th} and 10^{th} day. At the later stage of decay (remains stage) their larvae became dominant underneath the cadavers. In the building, the *H. illucens* was seen on the 7^{th} day and their 1^{st} larval instars on day 13^{th}. During the dry season, they arrived during the wet decay at the forest while only the larvae were seen in the building during the remains stage. The larval growth of *H. illucens* at both sites during dry season was poor while *S. excellens* and *S. analis* sharing the same family with *H. illucens were* seen at the outdoor only during the rainy season and were not found during the dry season at both locations. Thus, it was believed from the study that *H. illucens* as cosmopolitan wasp-mimicking flies is useful in forensic entomology in both seasons while the *Sagaricera* species will be evidence of rainy season and outdoor environments. The forensic significance of *H. illucens* is well documented in Lord *et al*. (1994) as insect that can breed in older carcasses and corpses.

The ulidiid flies is another important cadaveric insect of this region. Their arrival was consistent and predictable in both sites and throughout the seasons. The adults do arrive

during the bloating stage while the larvae do appear during the wet decay. It was observed from the study that the larvae usually appear when the first batch of blowflies have emerged and will continue to be found till the remains begin to degenerate. This observation concured with the reports of Abajue *et al*. (2013) that ulidiid larvae have similar potentials with the *H. illucens* larvae in forensic entomology and Abajue *et al*. (2014) that they can assist forensic investigators estimate the PMI of badly decomposed cadaver.

Similarly, cadaveric beetles constitute the guilds of predators as well as necrophages. Thus, the arrival of the predators mainly the histerids synchronizeed the presence of necrophagous flies while the presence of necrophegous beetles characterized the peak of wet decay, dry decay and remains stages, depending on the seasonal variation. Among the beetles of this study in relation to forensic entomology include the clerids, demestids, histerids and staphylinids.

For the clerids, they are dominated by cosmopolitan *Necrobia* species of stored – product pests (Smith 1986). This study recorded the species of *N. ruficolis* and *N. rufipes* throughout the study period at both study locations. Their arrival in this work coincided with the wet decay stage of the cadavers. They formed the conspicuous insect group during the dry and remains decay stages. Their larvae also characterise the remains decay especially in the building.

The dermestid beetle observed in this study was *Dermestes frischii*. The beetles were found on the surfaces of the cadavers mainly on the hairs during the onset of wet decay throughout the study period but took residence on the cadaveric tissues during the dry or remains stages.

During the rainy season at both sites, the beetles disappeared earlier during the mid remains decay. This study assumed that the cause was due to the absence of dry decay stage which usually constitutes their "meal". Hence, being true keratophagous beetles that "meal opportunity" was sharply denied which resulted to their poor oviposition and development hence, leading to their disappearance earlier than what was observed during the dry season. Thus, during the dry season, they constituted the necrophagous group of beetles that consumed the greater portion of the dried cadavers. Dermestid beeltes have global recognition in forensic entomology, may be due to their synanthropy as stored product pests. However, it seemed that the species are geographically and seasonally influenced. Thus, this study recorded a singleton of *D. frischii* throughout the study period at both study locations.

The histerids of this study include *Hister monitor, H. castanipes* and *Hister* sp. All the species are most common on the cadavers during the rainy season while the *H. castenipes* dominated during the dry season. It was observed from the study that the species were predators of calliphorid larvae irrespective of the season. The arrivals of histerid beetles were persistent and predictable, precisely on the 2nd day of the carrions' decomposition at both locations and through the study period. The predictability of arrival on the cadavers was the reason for their inclusion as insects of forensic importance which reflected the reports of (Smith, 1986; Amendt *et al.,* 2004; Gennard, 2007; Byrd and Castner, 2010).

The staphylinids recovered in this study included the *Philonthus* sp. and *Staphylinus* sp. While the *Staphylinus* sp. was recovered once at the forest during the rainy season, the *Philonthus* sp. was collected throughout the study period at the forest. None of the species were recovered from the cadavers in the building. These species are among the guild of predators

reported as predators of immature stages of calliphorid flies hence, their forensic importance (Braack, 1986).

In the case of hymenopterans (ants), the groups recognized were mainly omnivores and predators. The arrivals of the ants on the cadavers were not often predictable and hence play more of ecological functions. However, the group that predated on fly eggs and larvae can alter forensic result while the group that bite cadavers may mimic abrasions of skin often times mistaken for signs of poisoning from sulphuric acid that had been running down the chin and neck (Benecke, 2004). The ants of this study were mainly the formicids which included: *Camponotus acvapimensis, C. maculatus, C. perrisi, Dorylus affinis, Oecophyla longinoda and Pheidole* sp. Among the species, *C. aevapmensis, C. maculatus* and *C. perrisi* constitute the group of predating ants that can alter forensic result. While the *C. maculatus* were actively nocturnal ants predating on eggs and larvae of flies, the *C. perrisi* were actively diurnal, predating on the adult calliphorid flies, their eggs and their larvae. The foraging behavior of *C. perrisi* is interesting as they do form linear gregarious group while taking flies and their larvae to their hole(s). *C. acvapmenis* and *O. longinoda* also predated on either the eggs or the larvae. However, the overall influence of the two ants on the cadaver community was minimal. In addition, *D. affinis* usually form a colony in between the larval and pupal mass of calliphorid flies. They took residence in between the larval mass in the building but could not repeat such at the forest during the dry season. The behavior was assumed to be because of presence of other ants recorded at the same time at the forest. Hence, the study further assumed that *D. affinis* is associated with environment that is moist and less competitive with other ants. Interestingly, the presence of *Pheidole* sp. in this study is a

reflection that they are synanthropic ants as reported by (Ewuim and Osondu, 2008). They were assigned omnivorous role in this study as they were only found in the building actively feeding on the cadavers as well as on the immature stages of the flies. Except *D. affinis* and *Pheidole* sp. the other ants were restricted to the forest, suggesting that their recovery is a proof of outdoor exposure.

5.1.2 Ecological classification of the cadaveric community

The insects recovered from the cadavers showed similarity with carrion communities of other regions at the family level, but distinctive at the species level. The strong phylogenetic trends underlying the ecological characteristics of the community members at the family level fortunately means that the cadaver communities contained ecological analogs or surrogates of members of other regions. It is therefore possible to generalize the ecological structure of terrestrial invertebrate carrion communities globally by describing them in terms of the ecological roles filled by the animals. These roles or functions are termed community guilds (Villet, 2011). Hence, Smith (1986) grouped these guilds into necrophages, predators and parasites, omnivores and adventives species.

The necrophagous insects absolutely are obligate carrion eaters (Villet, 2011). They constitute the most abundant, widely recognized, well studied and useful guild for calculating the age of a corpse. The reason is because of their predictability on cadavers. The principal members of the guild in this study include the calliphorids, ulidiids, stratiomyids, clerids and dermestids. These guilds constituted the wet and dry feeders which absolutely differentiated the flies and their larval feeding on moist decaying tissues from the beetles that usually feed on desiccated

and keratinized tissues. The wet feeders are the best for calculating the PMI of cadavers (Villet, 2011).

In the same vein, predators and parasites of this study were guilds that include the histerids, staphylinids and ants that predated specifically on necrophagous insects. Payne (1965) placed the larvae of *C. albiceps* in this category while Braack (1986) stated that the *Necrobia* and *Dermestid* species may prey on maggots, but is not significant to group them as predators because the "maggot meal" is not their primary diet. Hence, such facultative predators are suggested to be recognized as omnivores (Van Laerhoven, 2010). Similar observation was made in this study between the *N. rufipes* and *D. frischii*. The 1st larval instar of *D. frischii* was seen being attacked by *N. rufipes* adult. Hence, the study opined that *N. rufipes* were neither predators nor omnivores thus suggesting that such behavior is a competitive traits geared towards establishing dominance on the cadaver community. On the contrary, the members that are forensically recognized as parasitoids were not recovered in this study. Hence, it was assumed from the study that factors that present the parasites on cadavers were not elucidated in the region which is essentially a mesophilic in nature.

The omnivorous insects of this study were the group that fed on any resource of the cadaver community. In this study, *Pheidole* sp. and *D. affinis* were recorded in this group. However, few cockroaches (Blatoidae) also recovered were included in this guild. These groups of omnivorous species were also reported by Ekanem and Dike (2010). It was also assumed from the study that the presence of the omnivores on the cadavers have the potentials to disrupt the activities of the necrophages which may mar the forensic importance of the cadaveric community.

Furthermore, the adventive groups of this study were mainly arthropod relatives other than the insects. Their arrivals on cadavers were unpredictable. The forensic importance of this group according to Smith (1986) is that they may help to associate corpses with places or suspects by providing trace evidence. Hence, they describe the habitat. In this study, adventives recorded include; ispods, millipedes and arachnids.

5.1.3 Succession pattern of the insects recovered from the cadavers

The predictable occurrence of insects on cadaver as it decomposes is termed succession. Though the decomposition may not be stage dependent, there is a unique pattern of change that must be explained which involves initial colonization of the cadaver by wet necrophages as well as their predators. As the wet tissues consuming necrophages leave, the dry necrophages become progressively and numerically dominant. This trend will continue until the core keratophagous insects are left to exhaust the cadaver resource.

Villet (2011) opined that dietary differences between species affect the rate at which necrophagous larvae can grow. The group that abundantly eat and digest soft tissues will develop faster and disperse earlier than those that arrive at the same time but eat less nutritious tissues, creating the impression of a succession. It was therefore suggested from this study, that this was the reason the clerid and dermestid beetles found during the onset of the wet decay became progressively and numerically dominant during the dry decay and the remains stages. Hence, they ate keratinized meal which was less nutritious than the soft tissues consumed by the flies.

In this study, the succession pattern of the insects recovered from the decomposing cadavers is unique to this region. The study recognized wet and dry decay stages as against what other researchers recognized as active decay and advanced decay stages. It was believed from this study that the difference is a matter of choice of adjective which conveniently enhanced individuals' interpretations of the stages of decomposition. Thus, this study chose the wet and dry decay stages to enhance the understanding of the cadaver decomposition during the two seasons (dry and wet) of the region which the study passed through. Thus, the succession from fresh to remains was distinctively unique with at least one insect species that distinctively mark each stage of the decomposition. Hence, during the fresh stage, the cadavers were dominated by calliphorid flies. The ages of these immature insects were the anchor points that gave the minimum estimation of time of death. During the bloating stage, the calliphorids presence was still forensically important. In addition to that, was the presence of histerid beetles that arrived to predate on the blowfly larvae. During the wet decay, the presence of the two insect families (calliphorids and histerids) still played baseline support. Thus, the estimate of the age of the cadavers at that stage was focused on the clerid and dermestid beetles and was characteristically marked by the presence of ulidiid larvae.

Sequentially, the dry decay stage was not observed in this study during the rainy season hence, the remains stage was observed immediately after the wet decay. The larvae of stratiomyiid flies, dermestid beetles as well as absence of calliphorid immature stages, estimated the age of the cadavers. Similar observation repeated itself at both locations with little or no difference between the two seasons. The chronology of these insects and the procedure of their succession on the decomposing cadavers were the biological clocks

adopted by earlier researchers in forensic entomology, to conveniently estimate the age of a corpse or cadaver. Hence, the succession table of the cadaveric insects of this region has been developed for this task (figs 4.8; 9; 10; and 4.11). Identifying particular or group of insects at different stages of cadaver decomposition with a view to estimating PMI of the cadaver is the hall mark of forensic entomology and is globally documented (Smith, 1986; Benecke, 2004; Amendt *et al*., 2004; Gennard, 2007; Byrd and Castner, 2010; Villet 2011).

5.2 Evaluating the Interaction between the Cadavers and the Micro-environmental Factors

The overview of ecological theory in relation to carrion community and its spatial variation components such as geographical distribution and habitat preference is copiously reviewed by VanLaerhoven (2010). Allogenic communities such as cadaver community in every occasion changes over three time scales which Villet (2011) recognized as circadian, annual and the duration of decomposition. In this study, rainfall was observed to cause the recognized differences in the decomposition of the cadavers between rainy season and the dry season. However similar environmental conditions affected the two study locations (building and forest). It was observed from the study that almost daily rainfall during the rainy season, decreased the temperature and made the atmosphere wet. Therefore, rainfall is believed to be one of the major physical factors that affected the degree of hotness or coldness of the atmosphere as well as the moisture content of the region.

The succession patterns of the insects recovered at the forest in this study showed that the dipterans peaked during the initial stages of decomposition (bloated, wet and early remains)

during the rainy season. During the dry season, the stages that contained them were (bloated, wet, dry and early remains). However, the coleopterans appeared to dominate the dry and remains decay stages. In addition to the dominance of coleopterans at these stages were the presence of stratiomyiid and ulidiid flies which were consistent in this study irrespective of the season and the location of the cadavers. This pattern of succession is in line with the reports of Wolff *et al.* (2001) and Clery, (2001). The calliphorid flies in the genus *Chrysomya* recovered in this study were truly African documented blowflies (Williams and Villet, 2006a) and have been repeatedly reported in Nigeria (Ekanem and Dike, 2010, Ekrakene and Iloba, 2011; Abajue *et al*., 2013 and 2014). In addition to the calliphorid flies; the stratiomyiid and ulidiid flies made the list of flies that are of forensic importance to this study, whereas the dermestid, clerid, and the histerid beetles are the only beetles this study can claim their forensic importance. The beetles were also collected on pig carrions decomposition in Okija, Anambra State by Abajue *et al*. (2015). The slight difference in the beetles of forensic importance is attributed to the irregular arrival of other beetles of this study. However, the staphylinids seem to appear regularly, but not predictable and their predating potentials are very minimal.

In the building, it was observed that there was slight access delay of the calliphorid flies who were the first colonizers of this study for at least six (6) hours for both seasons. This contradicted the findings of Amendt *et al*. (2004) and Goff (1991) who reported delay of about three (3) days.

Relative to indoor decomposition, the ability of *H. illucens* to have access into the building through the narrow openings is interesting to note. This fly has been noted to be found at

compost heaps especially if animal origin is associated with the compost and its presence on cadaver as an evidence of older cadaver decomposition (Lord *et al*., 1994). In this study about 2 or 3 of this fly were found during wet decay and its larvae become preponderant during the remains stage. This observation was similar to the reports of Nazni *et al*. (2011) who observed the larvae at the indoor location in Malaysia between day 29 and day 86 when monkey carrions had mummified. In this study however, it was submited that only the larval stage of *H. illucens* which were recovered during the wet, dry and remains decay stages have forensic potentials because of the behavioural disposition of the adults. When the adults arrived on the cadaver, they tend to perch on the interface of the cadavers and walk towards under the body or any other debris around the body to lay their eggs. When the larvae emerge, they tend to avoid the cadaver like the adults and concentrated under the cadaver for their development. Hence in this study, it was attributed that such behaviour is for survival concealment which is beneficial to their survival because at the time of their larval appearance, the calliphporid larvae and their associated predators have dominated every part of the cadaver. Hence, they tend to succeed the calliphorid larvae and their predators during the remains stage, when predation and competition is not active. Similar behavioural disposition was consistently observed also with the *Chrysomyza africana*. Numerically, the diversity of insects recovered in the simulated building was slightly lower than the insects recovered at the forest cadavers. The insect at the forest is higher because it is their natural abode. However, many of the insects recovered are not of forensic importance hence, many of them played roles as predators, adventives and incidentals. This observation agreed with the reports of Goff (1991) who observed more insect species at the outdoor than in the indoor.

Micro-environmentally, it was suggested that temperature and relative humidity at the forest and in the building do not differ during each season. Hence, the cadaver's decomposition and insects of forensic importance recovered on them appeared to be the same throughout the study period. Interestingly, cadaveric insects such as flies and beetles are diurnal insects. Thus, while the flies fed on the cadaver's fluids and oviposited on them, some of the beetles only fed on the cadaver's tissues and their associating fauna and conduct their reproductive cycles elsewhere. Beetles such as clerids and dermestids which fed on dry parts of the cadavers as well as the histerids that predated on fly larvae took residence on the cadavers during the night hours.

Ecologically, environmental temperatures and light levels are implicated as the principal mechanisms driving circadian cycles (Villet *et al*., 2010). It was observed from the study that the significant difference between the temperatures and relative humidity during the two seasons did not influence the activities of cadaveric insects especially their immature stages. The light levels observed during the study period between the two seasons do not differ but however, prompted the disappearance of the flies during the night period. In addition, the clerids and the dermestids that took residence on the cadavers during the day, where less active during the night. Villet *et al*. (2010) argued that circadian rhythms also affect the migration time of mature maggots from carrion and the eclosion of adults. That assumption was to some extent not validated by this study because the temperature and relative humidity recorded in the study fluctuated between 23°C and 37°C which is siginficantly mesophilic that favours daily and yearly survival and development of insect species in the tropics. However, the findings of this study agreed in some points with Villet (2011) that occurrence of circadian

cycles in adult flies (calliphorids and sarcophagids) is important in forensic contexts, because it constrains the hours when eggs are laid and this affects the estimations of PMIs. The reason is that when death occurs in the late evening, flies will probably not lay eggs on it until the next day. Thus, an estimate of the PMI based on the development of the immature flies will need to take this condition into account.

While circadian cycles have minor effects on the presence of immature stages of cadavers associated insects, seasonal cycles affect whether a species breeds on it at all. Thus, core membership of the carrion community is therefore more strongly influenced by seasonal variation than by time of the day (Villet, 2011). With reference to that, *Sagaricera analis* and *S. excellens* (Stratiomyidae) were found to breed on the cadavers during the rainy season at the forest and not in the building or during the dry season. Thus, the forensic implication of this observation is that the presence or absence of some carrion associated insects may provide evidence of the time of the year when the carrion died or when they were colonized.

In addition, insects associating with decomposing carcasses usually change at a predictable time forming what is termed succession (Smith 1986; Tantawii *et al.*, 1996). However, because the allogenic community is ephemeral, it thus lacks pioneering primary producers and lacks a climax or mature community. Thus, it is referred to as micro community (Villet, 2011).

It was therefore affirmed from this study, that truly the community lacked primary producers and the decomposition process was progressively successive without climax community. Therefore, in as much as there were micro-envirnmental differences between the two study

locations and between the two seasons; the differences however, never produce meaningful effect on the cadaver's colonization as well as on the insects larval developments which were the basis in forensic entomology. Hence, all the micro-environmental parameters evaluated with insect larvae to elucidate their effects on the cadaver community failed and thus validated the above assumption.

5.3 Toxicological Influence of the Zinc Phosphide Poison on the Cadaveric Fauna

Cadaver tissues are consumed by cadaveric insects, and it is possible they will consume any poison in the cadaver too. If toxic substances are present, they may be metabolized or stored in their tissues or in their cuticles. Amendt *et al.* (2004) stated that larvae which feed on corpse may sequester drugs and toxicants which had been ingested by the dead person.

It is thus affirmed in this study that toxins ingested by deceased person can be detected on insects larvae recovered on the body when the body is badly decomposed and when matrices such as blood, urine and soft tissues were no longer viable for toxicological analysis. Hence, zinc which was one of the active ingredients in the commercially produced zinc phosphide as a rodenticide and sold with the trade name, "Commando" without restriction in Nigeria which was used to sacrifice the pigs was assessed in the fly larvae recovered on the cadavers. However, the phosphorus which was also one of the active ingredients was not detected. Therefore, when a decomposing body is difficult to examine for toxicologically relevant substances due to lack of appropriate sources such as tissue, blood or urine, immature stages of insects on the body such as larvae and pupae can serve as alternative materials. In affirmation, reports of (Nolte *et al.,* 1992; Goff and Lord, 1994 and 2001; Introna *et al*., 2001

and Campobasso *et al*., 2001) agreed that analyzing insects recovered from cadaver may enable toxicological assessment of the cause of death.

Therefore, the detection of heavy metal such as zinc in the larvae of various species of calliphorids, stratiomyiids and ulidiids in this study affirmed the reports of Nourteva and Nourteva (1982) and Gunatilake and Goff (1989) who detected mecury and malathion pesticide respectively from the larvae of calliphorid flies. Similar studies of (Rashid *et al*., 2008; Liu *et al*., 2009; Bakr *et al*; 2012) have also validated the presence of pesticides and metals on fly larvae recovered on decaying carcasses.

Presently, the role of drugs or poisons in insect larvae are not clearly understood (Gennard, 2007) and is not known how larvae bioaccumulate or eliminate drugs or poisons and how they affect larval development (Gautam *et al*., 2013). However, previous reports have shown that different categories of drugs of abuse affected the growth of fly larvae encountered on decomposing corpses believed to have died as a result of the abuse or over dose, by increasing the rate of the larval development (Goff *et al*., 1989; 1991; O'Brien and Turner, 2004). Contrary reports also showed that underestimation of PMI up to twenty-four (24) hours is possible if the presence of morphine in tissue is not considered when calculating the development time of *Lucilia sericata* (Bourel *et al*., 1999). In another case study, Gunatilake and Goff (1989) stated that the presence of malathion pesticide caused underestimation of three (3) days because the developmental stages of calliphorid flies used to calculate the PMI of the deceased indicated PMI of five (5) days while the victim was last seen alive eight (8) days prior to the discovery of the body.

In a nutshell, some of the empirical studies of (Introna *et al*., 1990; Goff *et al.*, 1997; Hedouin *et al.,* 2001; Pien *et al.*, 2004) and few case studies of (Kintz *et al.,* 1994; Sadler *et al.,* 1995) are obvious that cadaveric insects are useful in toxicological assessments known as entomotoxicology in forensic entomology. However, having stated that the role of drugs in insects recovered from carrions are yet to be ascertained, some reports observed that some drugs caused rapid larval development Goff *et al.* (1989 and 1991) while some pesticides retard the larval growths (Bourel *et al.*, 1999; Gunatilake and Goff 1989). In this study however, developmental variations were not observed in relation to the linear growths of the calliphorid, stratiomyid and ulidiid larvae that fed on the poisoned pig cadavers as compared with those from the non-poisoned pig cadavers. Therefore, the presence of zinc and absence of phosphorus in the insect immature stages (larvae and pupae) is an indication that at least one of the components of the "zinc phosphide" poison metabolised in the decomposing pig cadavers but did not alter the cadaveric decomposition and insect larval development. This conformed in parts to the reports of Ekrakene (2012) who detected monocrotophos metabolites on the puparіae and adult flies recovered from monocrotophos poisoned pigs at Benin, Nigeria. Quantitatively, the zinc detected in the fly larvae and pupae was not of lethal dose. It is noteworthy, that metabolisms of pesticides are usually rapid. Thus, the zinc phosphide rapidly metabolized in the cadaver body liberating the phosphorus into phosphine gas which caused acute kidney, lung and heart failures. This biochemistry was the reason the phosphorus component was not detected, in addition to that, phosphorus is ordinarily a free radical element that is not stable in the body. The zinc on the other hand, is a heavy metal nutritionally essential for animal health in small quantity but large amount causes acute or chronic toxicity or poisoning. Thus, heavy metals such as zinc become toxic when they are

not metabolized by the body and accumulate in the soft tissues (Ezeonu, 2015). The acute toxicity of the zinc phosphide which caused the death of the pigs as well as the non-metabolism of the zinc on the pig led to its recovery from the insect larval tissues. From the perspective of entomophysiology, the poison does not in any way influence the growth rate of the larvae recovered from the poisoned pig cadavers. Hence, in reference to the recommended dietary allowance RDA of zinc in U.S., 8 mg/women and 11 mg/men is recommended (Bales and Ritchie, 2009) thus, pointing that zinc is nutritionally essential in minute quantity. Apparently, no standard recommendation of zinc and phosphorus is documented for insects. Therefore, the zinc detected may not be reviewed with reference to quantity as regards to dosage on the insect larvae. Thus, the assumption is still based on comparism with the control samples which the quantity detected was lower than the test samples.

Interestingly, Gosselin *et al*. (2011) reviewed that one of the problems of entomotoxicology is interpretation of results which he attributed to experimental set up and lack of experimental validation. Thus, the differences observed in view of the cadaver decomposition, colonization and development was as a result of rainfall which differentiated the two seasons and not as a result of the killing agents or methods. In addition, conditions such as diapause as a result of physiological changes occasioned by temperature may not be cited in mesophilic environments such as the region of this study.

Conclusion

Heuristically, insects associated with the poisoned and asphyxiated pig cadavers have validated their usefulness in forensic and toxicological investigation relating to questionable deaths. Seasonal micro-environmental factors of this region slightly influenced the decomposition rates of the cadavers but did not affect the community composition, the guild structure and the community dynamics of the associated insects. The systemic poison such as rodenticicde (zinc phosphide) did not affect the decomposition rates of the cadavers or influenced the insect fauna succession and larval development.

Recommendations

The findings of the study are implicit; hence the study recommends that:

1. the succession patterns of insects associated with indoor and outdoor cadavers of this study can serve as baseline data for forensic entomology of this region, which proves to be a valuable tool in the investigation of questionable deaths;

2. the toxicological analysis in relation to badly decomposed corpse infested with insects and suspected to die of poison can be validated by analyzing the insect larvae especially when the toxicological matrices of the corpse are lacking;

3. the information contained in the study will be useful to police, and other law enforcement agencies as well as other coronary investigators on homicide case in Nigeria, to estimate the PMI of a questionable death while further research/ or

collaboration is required, for full potentials of this emerging discipline to be recognized in Nigeria.

Challenges of the study

According to Ezeonu (2015), Nigerian laboratories are poorly equipped and lack the requisite instruments for identifying poisons.

Lack of requisite instruments to assess the poison profiles of the sampled insects nearly sidelined this important aspect of the research.

Let's believe as Ezeonu (2015) stated, that biochips and biosensors are offering solutions for the future because of a recently developed biosensor in China which was able to detect the caloric contents of foods including fruits. Therefore, if mechanism of such sensor is developed for detecting poisons in animal tissues, will fast track forensic studies.

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Appendix 1

Tables subjected to Anova

larval length in (mm) of Calliphoridae on indoor poisoned cadaver during the rainy season

	Collection Days					
Observation (Replications)	4	5	6	7	F_{cal}	$F_{tab(0.05)}$
1	15.0	16.2	19.5	17.0		
2	13.8	16.8	18.8	17.8		
3	14.0	17.0	18.2	18.0		
4	14.1	17.1	18.0	19.0		
5	14.1	17.8	17.7	18.1		
Total	**71.0**	**84.9**	**92.3**	**89.9**		
Mean±Sem	**14.20**±0.21	**16.98**±0.26	**18.46**±0.32	**17.98**±0.32	33.3*	3.06

Analysis of variance of length of blowfly larvae recovered from the poisoned pigs in the simulated building in Awka, Nigeria from June to September, 2014

Source of variations	**Degree of freedom**	**Sum of squares**	**Mean of squares**	**Fcal**	**Ftab (0.05)**
Total	19	606.3			
Measurement	4	544.8	136.2	**33.3***	**3.06**
Error	15	61.5	4.1		

*Significant difference (P< 0.05)

Solving for the data above

$$SS = \Sigma x^2 - \left(\frac{\Sigma x}{n}\right)^2$$

$$TSS = TrtSS - ErSS; TrtSS \equiv Observation$$

$$TSS = 15.0^2 + 13.8^2 + 14.0^2 + \cdots + 18.1^2 - \frac{(3381)^2}{20}$$

$$TSS = 606.295$$

$$TrtSS = \frac{71.0^2}{5} + \cdots + \frac{89.9^2}{5} - \frac{(3381)^2}{20}$$

$$TrtSS = 544.815$$

$$ErSS = TSS - TrtSS = 606.295 - 544.815$$

$$ErSS = 61.48$$

Note: TSS, total sum of squares; TrtSS, treatment sum of squares; ErSS, error sum of squares.

For Indoor asphyxiated Cadaver-Rainy Season
Collection Days

Observation (Replications)	4	5	6	7	F_{cal}	$F_{tab(0.05)}$
1	15.8	16.9	17.9	19.0		
2	15.1	15.0	19.2	19.1		
3	15.0	15.0	19.5	20.0		
4	15.5	15.5	18.0	20.0		
5	15.0	15.6	18.8	20.0		
Total	**76.4**	**77.4**	**93.4**	**98.1**		
Mean±Sem	**15.28±0.16**	**15.48±0.35**	**18.68±0.32**	**19.62±0.23**	38.3*	3.06

$TSS = 805.55; TrtSS = 733.73; ErSS = 71.81$

Analysis of variance of length of blowfly larvae recovered from the asphyxiated pigs in the simulated building in Awka, Nigeria from June to September, 2014

Source of variations	**Degree of freedom**	**Sum of squares**	**Mean of squares**	**Fcal**	**Ftab (0.05)**
Total	19	805.6			
Measurement	4	733.7	183.4	**38.3***	**3.06**
Error	15	71.8	4.8		

*Significant difference (P< 0.05)

For Outdoor poisoned cadaver- Rainy Season

Days of Collections

Observation (Replications)	3	4	5	6	F_{cal}	$F_{tab(0.05)}$
1	15.5	16.6	15.1	16.2		
2	16.0	15.8	14.2	15.3		
3	15.0	16.9	13.1	_		
4	16.5	17.1	12.4	_		
5	15.2	15.8	_	_		
Total	**78.2**	**82.2**	**54.8**	**31.5**		
Mean±Sem	**15.64±0.27**	**16.44±0.27**	**13.70±0.58**	**15.75±0.28**	6.3*	3.36

$TSS = 251.44; TrtSS = 174.95; ErSS = 76.49$

Analysis of variance of length of blowfly larvae recovered from the poisoned pig at the forest in Awka, Nigeria from June to September, 2014

Source of variations	Degree of freedom	Sum of squares	Mean of squares	Fcal	Ftab (0.05)
Total	15	251.4			
Measurement	4	174.9	43.7	**6.3***	**3.36**
Error	11	76.5	6.9		

*Significant difference (P< 0.05)

For Outdoor asphyxiated cadaver- Rainy Season

Collection Days

Observation (Replications)	3	4	5	6	F_{cal}	$F_{tab(0.05)}$
1	13.8	14.2	17.9	15.2		
2	14.5	15.9	17.8	15.5		
3	14.0	17.5	17.2	_		
4	14.0	16.0	17.0	_		
5	15.2	16.7	15.2	_		
Total	**69.5**	**80.3**	**85.1**	**30.7**		
Mean±Sem	**13.90±025**	**16.06±0.55**	**17.02±0.49**	**15.35±0.09**	6.6*	3.26

$TSS = 373.306; TrtSS = 257.056;\ ErSS = 116.25$

Analysis of variance of length of blowfly larvae recovered from the asphyxiated pigs at the forest in Awka, Nigeria (from June to September, 2014)

Source of variations	Degree of freedom	Sum of squares	Mean of squares	Fcal	Ftab (0.05)
Total	16	373.3			
Measurement	4	257.1	64.3	**6.6***	**3.26**
Error	12	116.2	9.7		

*Significant difference (P< 0.05)

Indoor asphyxiated cadaver- Dry Season

Days of Collection

Observation (Replication)	4	5	6	7	F_{cal}	$F_{tab(0.05)}$
1	10.1	18.8	17.0	16.1		
2	10.0	19.4	18.5	15.2		
3	9.2	19.8	19.2	15.0		
4	8.5	19.8	18.2	15.8		
5	9.8	18.2	17.3	19.0		
Total	**47.6**	**96.0**	**90.2**	**76.3**		
Mean±Sem	**9.52±0.30**	**19.20±0.31**	**18.04±0.40**	**15.26±0.72**	115.2*	3.06

$TSS = 2{,}889.05;\ TrtSS = 2{,}797.975;\ ErSS = 91.12$

Analysis of variance of length of blowfly larvae recovered from the asphyxiated pig in the simulated building in Awka, Nigeria from January to April, 2015

Source of variations	**Degree of freedom**	**Sum of squares**	**Mean of squares**	**Fcal**	**Ftab (0.05)**
Total	19	2889.1			
Measurement	4	2797.9	699.5	**115.2***	**3.06**
Error	15	91.2	6.1		

*Significant difference (P< 0.05)

Indoor poisoned cadaver- Dry Season

Days of Collection

Observation (Replications)	4	5	6	7	F_{cal}	$F_{tab(0.05)}$
1	12.5	18.2	17.2	17.0		
2	11.5	13.8	16.0	19.2		
3	11.1	14.1	17.2	19.0		
4	11.0	14.8	17.5	18.8		
5	_	13.4	16.2	17.2		
Total	**46.1**	**74.3**	**84.1**	**91.2**		
Mean±Sem	**11.53±0.31**	**14.86±0.87**	**16.82±0.30**	**18.24±0.47**	17.2*	3.11

$TSS = 1{,}340.64211;\ TrtSS = 1{,}114.24711;\ ErSS = 226.395$

Analysis of variance of length of blowfly larvae recovered from the poisoned pigs in the simulated building in Awka, Nigeria from January to April, 2015

Source of variations	**Degree of freedom**	**Sum of squares**	**Mean of squares**	**Fcal**	**Ftab (0.05)**
Total	19	1340.6			
Measurement	4	1114.2	278.6	**17.2.***	**3.11**
Error	14	226.4	16.2		

*Significant difference (P< 0.05)

Outdoor asphyxiated cadaver- Dry Season

Days of Collection

Observation (Replications)	3	4	5	6	F_{cal}	$F_{tab(0.05)}$
1	5.0	11.0	16.5	16.8		
2	6.0	13.0	15.8	17.2		
3	5.8	11.2	15.0	15.2		
4	5.2	12.4	15.0	15.8		
5	5.0	10.0	15.1	16.2		
Total	27.0	57.7	77.4	81.2		
Mean±Sem	5.40±**0.21**	11.54±**0.53**	15.48±**0.30**	16.24±**0.35**	124.7*	3.06

$TSS = 3{,}798.455;\ TrtSS = 3{,}687.535;\ ErSS = 110.92$

Analysis of variance of length of blowfly larvae recovered from the asphyxiated pig at the forest in Awka, Nigeria from January to April, 2015

Source of variations	**Degree of freedom**	**Sum of squares**	**Mean of squares**	**Fcal**	**Ftab (0.05)**
Total	19	3798.5			
Measurement	4	3687.5	921.9	**124.7***	**3.06**
Error	15	111.0	7.4		

*Significant difference (P< 0.05)

Outdoor poisoned cadaver- Dry Season

Collection Days

Observation (Replications)	3	4	5	6	F_{cal}	$F_{tab(0.05)}$
1	6.0	13.8	15.2	14.8		
2	5.5	12.8	15.3	17.2		
3	5.5	14.0	16.1	15.2		
4	7.0	13.2	16.5	16.2		
5	5.8	13.2	14.8	14.6		
Total	**29.8**	**67.0**	**77.9**	**78.0**		
Mean±Sem	**5.96±0.28**	**13.40±0.22**	**15.58±0.31**	**15.60±0.49**	128.1*	3.06

$TSS = 3{,}221.855;\ TrtSS = 3{,}130.255;\ ErSS = 91.60$

Analysis of variance of length of blowfly larvae recovered from the poisoned pig at the forest in Awka, Nigeria from January to April, 2015

Source of variations	**Degree of freedom**	**Sum of squares**	**Mean of squares**	**Fcal**	**Ftab (0.05)**
Total	19	3221.9			
Measurement	4	3130.3	782.6	**128.1**	**3.06**
Error	15	91.6	6.1		

*Significant difference (P< 0.05)

Appendix 2

Mean length of blowfly larvae (mm) from indoor asphyxiated against poisoned cadavers-rainy season (subjected to t-test)

Larval growth (mm)

Replication (Days)	Asphyxiated	Poisoned	T_{cal}	$T_{tab(0.05)}$
4	15.3	14.2		
5	15.5	16.4		
6	18.7	18.8		
7	19.6	18.0		
Total	**69.1**	**67.1**		
Mean±Sem	**17.3±1.10**	**16.8±1.01**	**0.1**^{NS}	**2.4**

Hypothesis:

Ho: Asphyxiated = Poisoned (the growth rates was not affected by the poison)

Ha: Asphyxiated ≠ Poisoned (the growth rate was affected by the poison)

Decision Rule: Reject Ho, if $t_{cal} \geq t_{tab\ (0.025)}$ and accept Ha.

$$t_{cal} = \frac{\bar{x}_1 - \bar{x}_2}{s_{\bar{x}_1} - s_{\bar{x}_2}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad \text{Step 1}$$

$\bar{x}_1 = 1st\ mean;\ \bar{x}_2 = 2nd\ mean$

$$S_{\bar{x}_1} = SS_1 = \Sigma x_1^2 - \frac{(\Sigma x_1)^2}{n}$$

$$= SS_2 = \Sigma x_2^2 - \frac{(\Sigma x_2)^2}{n} \qquad \text{Step 2}$$

Pooled Variance S^2p

$\frac{SS_1+SS_2}{n_1+n_2-2}$ ..... Step 3

$S_{\bar{x}_1}^2 - S_{\bar{x}_2}^2 = \frac{S^2p}{n_1} + \frac{S^2p}{n_2}$ ..... Step 4

$S_{\bar{x}_1} - S_{\bar{x}_2} = \sqrt{\frac{S^2p}{n_1} + \frac{S^2p}{n_2}}$ ..... Step 5

$\frac{\bar{x}_1-\bar{x}_2}{S_{\bar{x}_1}-S_{\bar{x}_2}}$.....Step 6

Solving for t tabulated

$t_{tab} = \frac{\alpha}{2}(n_1 + n_2 - 2)$

$\frac{\alpha}{2}$ *is a two tailed test* $= 0.025(4+4-2)$

$= 0.025(6)$

$t_{tab} = 2.4$

$t_{cal} < t_{tab}$

So, we fail to reject Ho.

Thus, Ho is accepted. Therefore, the growth was not affected by the poison.

$\frac{\bar{x}_1-\bar{x}_2}{S_{\bar{x}_1}-S_{\bar{x}_2}}$ Step 6

$i.e\ t_{cal} = \frac{0.3}{4.6} = 0.06 \cong 0.1$

Table for Asphyxiated against poisoned cadaver at the in indoor (Dry Season)

Larval growth rates

Replication (Days)	Asphyxiated	Poisoned	T_{cal}	$T_{tab(0.05)}$
4	9.5	11.5		
5	19.2	14.5		
6	18.0	16.8		
7	15.3	18.2		
Total	**62.00**	**61.00**		
Mean±Sem	**15.50±2.16**	**15.30±1.46**	**0.03^{NS}**	**2.4**

Table for Asphyxiated against Poisoned cadaver at the outdoor (Rainy Season)

Larval growth rates

Replication (Days)	Asphyxiated	Poisoned	T_{cal}	T_{tab}
3	13.9	15.6		
4	16.1	16.4		
5	17.0	13.7		
6	15.3	15.8		
Total	**62.3**	**61.5**		
Mean±Sem	**15.6±0.66**	**15.4±0.58**	**0.07^{NS}**	**2.4**

Table for Asphyxiated against Poisoned cadaver at the outdoor (Dry Season)

Larval growth rates

Replication (Days)	Asphyxiated	Poisoned	T_{cal}	T_{tab}
3	5.4	6.0		
4	11.5	13.4		
5	15.5	15.5		
6	16.2	15.6		
Total	**48.6**	**50.5**		
Mean±Sem	**12.2±2.48**	**12.6±2.27**	**-0.05^{NS}**	**2.4**

For asphyxiated cadaver at the indoor against outdoor (Rainy Season)

Larval growth rates

Replication (Days)	Building	Forest	T_{cal}	T_{tab}
4	15.3	16.1		
5	15.5	17.0		
6	18.7	15.3		
Total	**49.50**	**48.40**		
Mean±Sem	**16.50±1.10**	**16.10±0.49**	**0.09^{NS}**	**2.8**

For asphyxiated cadaver at the indoor against outdoor (Dry Season)

Larval growth rates

Replication (Days)	Building	Forest	T_{cal}	T_{tab}
4	9.5	11.5		
5	19.2	15.1		
6	18.0	16.2		
Total	**46.70**	**42.80**		
Mean±Sem	**15.60±3.05**	**14.30±1.42**	**0.01^{NS}**	**2.8**

For poisoned indoor against outdoor (Rainy Season)

Larval growth rates

Replication (Days)	Building	Forest	T_{cal}	T_{tab}
4	14.2	16.4		
5	16.4	13.7		
6	18.5	15.8		
Total	**49.10**	**45.90**		
Mean±Sem	**16.40±1.24**	**15.30±0.82**	**0.02^{NS}**	**2.8**

For poisoned cadaver at the indoor against outdoor (Dry Season)

Larval growth rates

Replication (Days)	Building	Forest	T_{cal}	T_{tab}
4	11.5	13.4		
5	14.7	15.5		
6	16.8	15.6		
Total	**43.0**	**44.5**		
Mean ±Sem	**14.3±1.54**	**14.8±0.72**	**-0.09^{NS}**	**2.8**

For Rainy Season against Dry Season result (irrespective of killing methods)

Larval growth rates

Killing method	Location	Rainy Season	Dry Season	T_{cal}	T_{tab}
spg	Building	6.0	15.4		
ppg	Building	13.4	14.3		
spg	Forest	15.5	14.3		
ppg	Forest	15.6	14.8		
	Total	**64.30**	**58.80**		
	Mean±Sem	**16.10±2.27**	**14.70±0.26**	**0.3^{NS}**	**2.4**

2.27 0.26

For poisoned against asphyxiated cadavers irrespective of locations

Larval growth rates

Location	Season	spg	Ppg	T_{cal}	T_{tab}
Building	Rainy	16.5	16.1		
Building	Dry	15.4	14.3		
forest	Rainy	16.4	15.3		
Forest	Dry	14.3	14.8		
	Total	**62.60**	**60.50**		
	Mean±Sem	**15.70±0.51**	**15.10±0.38**	**0.3^{NS}**	**2.4**

Appendix 3

Mean length of ulidiidae larvae recovered from the cadavers (subjected to Anova)
For indoor asphyxiated cadaver (Rainy Season)

Length (Reps)	Collection Days								
	8	9	10	11	1213		14	F_{cal}	F_{tab}
1	_	_	12.3	11.8	10.3	12.0	13.0		
2	_	_	8.9	12.0	12.5	12.8	12.8		
3	_	_	10.5	11.1	11.0	12.5	12.9		
4	_	_	11.2	11.8	12.0	_	15.0		
5	_	_	9.3	11.3	11.2	_	_		
Total			**52.2**	**58.0**	**57.0**	**37.3**	**53.7**		
Mean±Sem			**10.40±**	**11.60±**	**11.40±**	**12.40±**	**13.40±**	**6.2***	**2.96**

For indoor poisoned cadaver (Rainy Season)

Length (Reps)	Collection Days								
	8	9	10	11	12	13	14	F_{cal}	F_{tab}
1	_	_	12.0	11.9	12.5	12.8	12.9		
2	_	_	11.1	11.0	11.0	11.9	11.3		
3	_	_	11.8	12.0	12.0	12.6	12.8		
4	_	_	10.2	12.0	12.0	13.1	13.0		
5	_	_	11.3	11.2	11.2	12.9	13.3		
Total			**52.2**	**58.0**	**57.0**	**37.3**	**53.7**		
Mean			**10.4**	**11.6**	**11.4**	**12.4**	**13.4**	**6.5***	**2.87**

For Outdoor asphyxiated cadaver (Rainy Season)

Length Rep	Collection Days								
	8	9	10	11	12	13	14	F_{cal}	F_{tab}
1	9.8	12.4	11.5	10.8	_	12.1	11.3		
2	8.0	12.2	12.6	10.5	_	13.0	11.8		
3	8.6	11.5	11.5	_	12.3	11.9	12.6		
4	9.3	11.5	10.8	_	12.1	_	11.0		
5	8.9	11.5	12.3	11.5	_	10.6	11.2		
Total	**44.6**	**59.1**	**58.7**	**32.8**	**24.9**	**47.6**	**57.9**		
Mean ±Sem	**8.9 ±0.31**	**11.80 ±0.20**	**11.70 ±0.32**	**10.90 ±0.23**	**12.20 ±0.06**	**11.90 ±0.44**	**11.60 ±0.29**	**20.9***	**2.87**

For Outdoor poisoned cadaver (Rainy Season)

Length Rep	Collection Days								
	8	9	10	11	12	13	14	F_{cal}	F_{tab}
1	12.1	11.2	11.0	12.0	12.0	_	12.0		
2	11.6	12.0	12.2	12.5	12.0	_	12.0		
3	10.9	11.0	10.2	12.3	9.8	_	12.1		
4	_	12.5	11.6	_	10.1	_	12.0		
5	_	12.6	11.5	_	_	_	12.0		
Total	**34.6**	**58.9**	**56.5**	**36.7**	**43.9**	_	**60.1**		
Mean ±Sem	**11.50 ±0.30**	**11.80 ±0.35**	**11.30 ±0.43**	**12.20 ±0.13**	**11.00 ±0.59**	_	**12.00 ±0.02**	**1.8^{NS}**	**2.87**

For indoor asphyxiated cadaver (Dry Season)

Length Rep	Collection Days								
	8	9	10	11	12	13	14	F_{cal}	F_{tab}
1	_	11.5	13.0	13.2	13.2	12.1	_		
2	_	11.8	13.0	14.2	13.5	12.6	_		
3	_	11.0	13.5	12.8	13.5	_	_		
4	_	10.5	13.4	_	_	_	_		
5	_	_	12.8	_	_	_	_		
Total		**44.8**	**65.7**	**40.2**	**40.2**	**24.7**	_		
Mean ±Sem		**11.20 ±0.26**	**13.10 ±0.13**	**13.40 ±0.32**	**13.40 ±0.08**	**12.30 ±0.16**	-_	**15.7***	**3.26**

For indoor poisoned cadaver (Dry Season)

Length Rep	Collection Days								
	8	**9**	**10**	**11**	**12**	**13**	**14**	**F_{cal}**	**F_{tab}**
1	_	10.6	12.6	13.1	13.2	12.1	_		
2	_	11.0	13.1	12.5	13.5	12.6	_		
3	_	9.8	13.2	13.6	13.5	_	_		
4	_	11.3	13.3	13.2	_	_	_		
5	_	_	12.4	_	_	_	14.3		
Total		**42.7**	**64.6**	**52.4**	**27.3**	**24.3**	**14.3**		
Mean ±Sem		**10.6 ±0.29**	**12.9 ±0.18**	**13.1 ±0.20**	**13.7 ±0.08**	**13.7 ±0.16**	**14.3 ±00**	**23.3***	**3.36**

For outdoor asphyxiated cadaver (Dry Season)

Length Rep	Collection Days								
	8	**9**	**10**	**11**	**12**	**13**	**14**	**F_{cal}**	**F_{tab}**
1	9.8	10.8	11.0	12.0	11.8	11.5	11.2		
2	10.6	11.0	11.8	13.0	11.8	12.0	11.5		
3	_	10.9	11.0	12.0	12.1	11.2	11.8		
4	_	_	12.0	12.1	12.3	13.1	_		
5	_	_	12.0	12.1	_	_	_		
Total	**20.4**	**32.7**	**57.8**	**61.2**	**48.0**	**47.8**	**35.3**		
Mean ±Sem	**10.20 ±0.25**	**10.90 ±0.04**	**11.60 ±0.23**	**12.00 ±0.19**	**12.00 ±0.11**	**12.00 ±0.37**	**11.80 ±0.13**	**10.2***	**2.84**

For outdoor poisoned cadaver (Dry Season)

Length Rep	Collection Days								
	8	**9**	**10**	**11**	**12**	**13**	**14**	**F_{cal}**	**F_{tab}**
1	_	10.8	13.0	12.2	12.0	11.3	11.3		
2	_	10.1	12.0	12.3	11.8	11.2	11.5		
3	_	_	12.1	12.0	11.8	12.3	12.3		
4	_	_	12.0	12.0	11.2	_	11.6		
5	_	_	11.5	12.2	11.4	_	11.8		
Total	_	**20.9**	**60.6**	**60.7**	**58.2**	**34.8**	**58.5**		
Mean ±Sem	_	**10.5 ±0.22**	**12.1 ±0.22**	**12.1 ±0.07**	**11.6 ±0.15**	**11.6 ±0.27**	**11.7 ±0.19**	**7.8***	**2.87**

Appendix 4

For indoor asphyxiated against poisoned cadavers (Rainy Season) (For Ulidiidae t-test)

Replication (Days)	Spg	Ppg	T_{cal}	T_{tab} (0.05)
10	10.4	11.3		
11	11.6	11.4		
12	11.4	11.7		
13	12.4	12.7		
14	13.4	13.5		
Total	59.20	60.60		
Mean±Sem	11.80±**0.50**	12.10±**0.42**	-0.14^{NS}	2.31

Hypothesis

Ho: spg = ppg growth was not affected by the poison

Ha: spg ≠ ppg growth rate was affected by the poison

Decision: Reject Ho, if $t_{cal} \geq t_{tab}$ [$(T_{tab}) = \frac{\alpha}{2}\ (n_1 + n_2 - 2)$]

For outdoor asphyxiated against poisoned (Rainy Season)

Replication (Days)	Spg	Ppg	T_{cal}	T_{tab}
8	8.9	11.5		
9	11.8	11.8		
10	11.7	11.3		
11	10.9	12.2		
12	12.2	11.0		
13	11.9	_		
14	11.6	12.0		
Total	79.0	69.8		
Mean±Sem	11.30±**043**	11.60±**0.17**	0.2^{NS}	2.20

Solving for t tabulation

$t_{cal} = \frac{\bar{x}_1 - \bar{x}_2}{S_{\bar{x}_1} - S_{\bar{x}_2}}$ ..... Step 1

$$S_{\bar{x}_1} = SS_1 = \Sigma x_1^2 - \frac{(\Sigma x_1)^2}{n}$$

$$S_{\bar{x}_2} = SS_2 = \Sigma x_2^2 - \frac{(\Sigma x_2)^2}{n}$$

Step 2

Pooled Variance S^2p

$\frac{SS_1 + SS_2}{n_1 + n_2 - 2}$ ..... Step 3

$S_{\bar{x}_1}^2 - S_{\bar{x}_2}^2 = \frac{S^2p}{n_1} + \frac{S^2p}{n_2}$ ..... Step 4

$S_{\bar{x}_1} - S_{\bar{x}_2} = \sqrt{\frac{S^2p}{n_1} + \frac{S^2p}{n_2}}$ ..... Step 5

$\frac{\bar{x}_1 - \bar{x}_2}{S_{\bar{x}_1} - S_{\bar{x}_2}}$ ..... Step 6

$\bar{x}_1 - \bar{x}_2 = 12.1 - 11.8 = -0.3$

$SS_1 = 10{,}816 + 13{,}456 + 12{,}996 + 15{,}376 + 17{,}956$

$= 70{,}600 - 70{,}092.8 \div 10 = 50.7$

$SS_2 = 12{,}769 + 12{,}996 + 13{,}689 + 16{,}129 + 18{,}225$

$= 73{,}808 - 73{,}447.2 = 36.1$

$\frac{50.7 + 36.1}{5 + 5 - 2} = \frac{86.8}{8} = 10.9$

$\frac{10.9}{5} + \frac{10.9}{5} = 4.4$

$\sqrt{4.4} = 2.1$

$\frac{0.3}{2.1} = 0.14$

$t_{tab} = 0.025(8)$

For indoor asphyxiated against poisoned cadavers (Dry Season)

Replication (Days)	spg	Ppg	T_{cal}	T_{tab}
9	11.2	10.6		
10	13.1	12.9		
11	13.4	13.1		
12	13.4	13.7		
13	12.3	0		
14	0	14.3		
Total	63.4	64.6		
Mean±Sem	12.70±**2.14**	12.90±**2.21**	-0.1^{NS}	2.31

For outdoor asphyxiated against poisoned cadavers (Dry Season)

Replication (Days)	Spg	Ppg	T_{cal}	T_{tab}
8	10.2	0		
9	10.9	10.5		
10	11.6	12.1		
11	12.2	12.1		
12	12.0	11.6		
13	12.0	11.6		
14	11.8	11.7		
Total	80.7	69.6		
Mean ±Sem	11.50±**0.27**	11.60±**1.67**	-0.1^{NS}	2.20

For indoor asphyxiated against outdoor asphyxiated cadavers (Rainy Season)

Replication (Days)	Indoor	Outdoor	T_{cal}	T_{tab}
10	10.4	11.7		
11	11.6	10.9		
12	11.4	12.2		
13	12.4	11.9		
14	13.4	11.6		
Total	59.2	58.3		
Mean ±Sem	11.80±0.50	11.7±0.22	0.1^{NS}	2.31

For indoor poisoned against outdoor poisoned cadavers (Rainy Season)

Replication (Days)	Indoor	Outdoor	T_{cal}	T_{tab}
10	11.3	11.3		
11	11.4	12.2		
12	11.7	11.0		
13	12.7	0		
14	13.5	12.0		
Total	60.6	46.5		
Mean ±Sem	12.10±**0.42**	11.60±**2.34**	0.3^{NS}	2.36

For indoor asphyxiated against outdoor asphyxiated cadvers (Dry Season)

Replication (Days)	Indoor	Outdoor	T_{cal}	T_{tab}
9	11.2	10.9		
10	13.1	11.6		
11	13.4	12.2		
12	13.4	12.0		
13	12.3	12.0		
14	_	11.8		
Total	63.4	70.5		
Mean±Sem	12.70±**0.38**	11.80±**0.19**	0.6^{NS}	2.26

For indoor poisoned against outdoor poisoned cadavers (Dry season)

Replication (Days)	Indoor	Outdoor	T_{cal}	T_{tab}
9	10.6	10.5		
10	12.9	12.1		
11	13.1	12.1		
12	13.7	11.6		
13	_	11.6		
14	14.3	11.7		
Total	64.6	69.6		
Mean ±Sem	12.90±**0.57**	11.60±**0.24**	0.7^{NS}	2.26

For rainy season against dry season (irrespective of killing method

Killing method	Location	Rainy season	Dry season	T_{cal}	T_{tab}
Spg	Indoor	11.8	12.7		
Spg	Outdoor	11.7	11.8		
Ppg	Indoor	12.1	12.9		
Ppg	Outdoor	11.6	11.6		
	Total	47.2	49.0		
	Mean±**Sem**	11.8±**0.11**	12.3±**0.32**	0.5^{NS}	2.45

For indoor against outdoor (irrespective of season)

Killing method	Season	Indoor	outdoor	T_{cal}	T_{tab}
Spg	Rainy	11.8	11.7		
Spg	Dry	12.7	11.8		
Ppg	Rainy	12.1	11.6		
Ppg	Dry	12.9	11.6		
	Total	49.5	46.7		
	Mean±**Sem**	12.4±**0.26**	11.8±**0.05**	0.8^{NS}	2.45

Appendix 5

Mean length of Stratiomyiidae recovered from the cadavers subjected to Anova.

For indoor asphyxiated cadver (Dry Season)

Length measurement	Rep	Collection Days								
		9	**10**	**11**	**12**	**13**	**14**	**15**	**F_{cal}**	**F_{tab}**
	1	0	0	0	0	0	0	0		
	2	0	0	0	0	0	0	0		
	3	0	0	0	0	0	0	0		
	Total	**0**	**0**	**0**	**0**	**0**	**0**	**0**		
	Mean	**0**	**0**	**0**	**0**	**0**	**0**	**0**		

For indoor poisoned cadaver (Dry Season)

Length measurement	Rep	Collection Days								
		9	**10**	**11**	**12**	**13**	**14**	**15**	**F_{cal}**	**F_{tab}**
	1	0	0	0	0	0	0	0		
	2	0	0	0	0	0	0	0		
	3	0	0	0	0	0	0	0		
	Total	**0**	**0**	**0**	**0**	**0**	**0**	**0**		
	Mean	**0**	**0**	**0**	**0**	**0**	**0**	**0**		

For outdoor asphyxiated cadaver (Dry Season)

Rep	Collection Days								
	9	**10**	**11**	**12**	**13**	**14**	**15**	**F_{cal}**	**f_{tab}**
1	10.9	10.2	11.8	11.1	0	0	0		
2	11.1	10.0	10.0	0	0	0	0		
3	11.0	9.8	9.8	0	0	0	0		
Total	**21.8**	**30.0**	**31.6**		**0**	**0**	**0**		
Mean ±Sem	**11.00 ±0.06**	**10.00 ±0.12**	**10.50 ±0.64**	**11.10 ±00**	**0**	**0**	**0**	**1.7^{NS}**	**5.14**

For outdoor poisoned cadaver (Dry Season)

Length of measurement	Rep	Collection Days							
		9	**10**	**11**	**12**	**13**	**14**	**15**	**F_{cal}**	**F_{tab}**
	1		8.2	8.2	11.1	0	0	0		
	2		8.2	11.0	12.0	0	0	0		
	3		0	10.3	11.5	0	0	0		
	Total		**16.4**	**29.5**	**34.6**	**0**	**0**	**0**		
	Mean ±Sem		**8.20 ±2.73**	**9.80 ±0.84**	**11.50 ±0.26**	**0**	**0**	**0**	**7.5***	**5.79**

For indoor asphyxiated (Rainy Season)

	9	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
1	0	0	0	8.3	10.4	16.3	16.4	18.2	20.3	20.2	20.1	20.1
2	0	0	0	8.1	9.6	16.1	16.2	18.1	0	20.1	20.1	21.5
3	0	0	0	8.2	10.8	16.3	16.1	0	0	0	0	22.8
Total	**0**	**0**	**0**	24.6	30.8	48.7	48.7	36.3	20.3	40.3	20.1	64.4
Mean ±Sem	**0**	**0**	**0**	8.2 **±0.06**	10.3 **±0.35**	16.2 **±0.07**	16.2 **±0.09**	18.2 **±6.05**	20.3 **±6.77**	20.2 **±6.72**	20.1 **±6.70**	21.5 **±0.78**

Tcal = 8.999* T tab= 3.55

For indoor poisoned (Rainy Season)

	9	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
1	0	0	0	8.1	9.3	15.7	17.3	19.0	20.1	20.2	0	19.8
2	0	0	0	7.6	10.2	16.5	17.0	18.1	20.3	20.1	0	22.3
3	0	0	0	0	0	0	17.4	18.0	0	0	0	20.1
Total				15.7	19.5	32.2	51.7	55.1	40.4	40.3		62.2
Mean ±Sem	00	00	00	7.90 **±2.62**	9.80 **±3.26**	16.10 **±5.37**	17.20 **±0.12**	18.40 **±0.32**	20.30 **±6.73**	20.20 **±6.72**	00	20.70 **±0.79**

Tcal= 5.329* Ttab= 3.65

For outdoor asphyxiated (Rainy Season)

	9	10	11	12	13	14	15	16	17	18	19	20
1	0	0	0	0	0	20.1	19.8	22.1	20.3	21.3	21.2	20.3
2	0	0	0	0	0	19.5	22.0	21.6	22.6			22.6
3	0	0	0	0	0	20.0						
Total	**0**	**0**	**0**	**0**	**0**	59.6	41.8	43.7	43.7	21.3	21.2	42.9
Mean	**0**	**0**	**0**	**0**	**0**	19.9	20.9	21.9	21.9	21.3	21.2	21.5
±Sem						**±0.19**	**±0.90**	**±0.20**	**±0.94**	**±00**	**±00**	**±0.94**

Tcal= 3.9^{NS} Ttab= 4.10

For outdoor poisoned (Rainy Season)

	9	10	11	12	13	14	15	16	17	18	19	20
1		8.2	16.5	11.06	0	20.5	18.6	22.4	21.6	20.6	21.6	20.1
2		0	15.8	0	0	20.0	20.6	20.6	0	21.3	0	21.3
3		0	17.1	0	0	19.2	22.1	0	0	0	0	0
Total		8.2	49.4	11.06	0	59.7	61.4	43.0	21.6	41.9	21.6	41.4
Mean		8.20	16.50	11.10	00	19.90	20.50	21.50	21.60	21.00	21.60	20.70
±Sem		**±00**	**±0.38**	**±00**	00	**±0.38**	**±1.01**	**±0.73**	**±00**	**±0.29**	**±00**	**±0.49**

Tcal= 17.3* Ttab= 3.68

Appendix 6

For Stratiomyiidae (Subjected to t-test)

For indoor asphyxiated against poisoned cadavers (Rainy Season)

Rep(Days)	Spg	Ppg	T_{cal}	$T_{tab}(0.05)$
12	8.2	7.9		
13	10.3	9.8		
14	16.2	16.1		
15	16.2	17.2		
16	18.2	18.4		
17	20.3	20.2		
18	20.2	20.3		
19	20.1	0		
20	21.5	20.7		
Total	**151.2**	**130.6**		
Mean±Sem	**16.80±1.56**	**16.30±2.37**	**0.1^{NS}**	**2.13**

For outdoor asphyxiated against poisoned cadavers (Rainy Season)

Rep(Days)	Spg	Ppg	T_{cal}	$T_{tab}(0.05)$
12	0	11.1		
13	0	0		
14	19.9	19.9		
15	20.9	20.5		
16	21.9	21.5		
17	21.5	21.6		
18	21.3	21.0		
19	21.2	21.6		
20	21.5	20.7		
Total	**148.2**	**157.9**		
Mean±Sem	**21.20±3.12**	**19.40±2.46**	**0.4^{NS}**	**2.16**

For indoor asphyxiated against poisoned (Dry Season) Nil

For outdoor asphyxiated against poisoned cadavers (Dry season)

Rep(Days)	Spg	Ppg	T_{cal}	T_{tab}
9	11.0	0		
10	10.0	8.2		
11	10.5	9.8		
12	11.1	11.5		
Total	**42.6**	**29.5**		
Mean±Sem	**10.70±0.25**	**9.80±2.55**	**0.3^{NS}**	**2.57**

For indoor asphyxiated against outdoor asphyxiated cadavers (Rainy Season)

Rep(Days)	Indoor	Outdoor	T_{cal}	$T_{tab}(0.05)$
12	8.2	0		
13	10.3	0		
14	16.2	19.9		
15	16.2	20.9		
16	18.2	21.9		
17	20.3	21.5		
18	20.2	21.3		
19	20.1	20.7		
20	21.5	21.5		
Total	**151.2**	**148.2**		
Mean±Sem	**16.80±1.56**	**21.20±3.11**	**0.8^{NS}**	**2.14**

For indoor poisoned against outdoor poisoned (Rainy Season)

Rep(Days)	Indoor	Outdoor	T_{cal}	$T_{tab}(0.05)$
12	7.9	11.1		
13	9.8	0		
14	16.1	19.9		
15	17.2	20.5		
16	18.4	21.5		
17	20.2	21.6		
18	20.3	21.0		
19	0	21.6		
20	20.7	20.7		
Total	**130.6**	**157.9**		
Mean±Sem	**16.3±2.37**	**19.4±2.46**	**0.5^{NS}**	**2.14**

Table 6 and 7 Nil i.e sppg Indoor and Outdoor ppg indoor and Outdoor (Dry season

For indoor vs outdoor (irrespective of the season)

Killing method	**Season**	**Indoor**	**outdoor**	**T_{cal} T_{tab}**
Spg	Rainy	16.8	21.2	
Spg	Dry	_	10.7	
Ppg	Rainy	16.3	19.4	
Ppg	Dry	_	9.8	
	Total	**33.1**	**61**	
	Mean ±Sem	**16.6±0.06**	**15.3±0.93**	**0.1^{NS}2.78**

Appendix 7

Maggot mass against ambient temperature and relative humidity

Rainy Season

Forest **Building**

Days	Maggot mass temp	Air tempt	R/humidity	Maggot mass	Air	R/humidity
3	39.0	30.0	86	0	29.6	80
4	39.8	28.0	78	37.1	29.3	82
5	37.0	26.8	88	37.4	27.6	86
6	0	25.6	90	33.4	26.4	88
Mean±Sem	**38.60±9.67**	**27.60±0.94**	**85.50±2.63**	**36.00±9.04**	**28.20±0.75**	**84.00±1.83**

Dry Season

Days	Maggotmass temp	Air tempt	R/humidity	Maggot mass	Air	R/humidity
5	31.4	31.0	28	36.0	30.7	33
6	39.2	33.1	26	37.3	37.3	24
7	42.0	32.1	34	40.3	36.9	35
8	42.4	31.3	28	38.2	32.8	29
9	37.8	32.8	46	37.2	36.2	38
Mean±Sem	**38.60±2.55**	**32.10±0.47**	**32.40±0.1.73**	**37.80±0.90**	**34.08±1.60**	**31.80±2.43**

Temperature and relative humidity throughout the study (Rainy Season)

	Air temperature				Relative humidity	
	Indoor	Outdoor		Body	Indoor	Outdoor
1	28.3	26.9	31.2	30.1	79	86
2	29.7	27.3	29.1	28.2	81	85
3	29.6	30.0*	31.8	33.8	80	86
4	29.3	28.0*	33.2	31	82	78
5	27.6	26.8*	28.1	27.7	86	82
6	26.4	25.8*			88	88
7	27.5	26.6			80	83
8	28.1	27.1			71	77
9	27.7	26.4			84	80
10	28.3	28.5*			82	77
11	27.4	28.8*			82	81
12	25.2	24.8			82	81
13	24.3	20.1			93	96
14	25.2	27.0			86	81
15	21.3	21.6			90	96
16	29.3	27.0*			78	84
17	29.1	26.8*			79	82
18	28.5	27.3*			90	93
19	28.3	26.7			86	86
20	25.2	23.6			88	90
21	24.8	24.3			89	92
22	24.6	24.2			92	94
23	26.3	24.8			91	94
24	25.8	25.1			90	96
25	25.3	24.3			87	89
26	24.8	24.1			86	89
27	26.0	25.5			87	90
28	25.2	23.8			86	91
29	26.1	26.0			88	92
30	24.9	24.3			87	93
Mean±Sem	26.67±**0.36**	24.98±**0.34**	30.68±**0.34**	30.16±**0.45**	85±**0.90**	86.76±**1.06**

Dry Season

	Indoor	Body	R/H	Air Outdoor	Body	R/H
1	30.3	30.4	30	27.8	27	23
2	33.1	31.1	27	28.3	30.4	20
3	32.6	30.2	20	30.6	28.8	10
4	35.1	32.1	23	30.8	30.8	24
5	30.7	29.8	33	31	30	28
6	37.1	34.8	24	33.1	31.1	26
7	36.9	37.8	35	32.1	30.8	34
8	32.8	32.3	29	31.3	31.3	28
9	36.2	35.8	38	32.8	31.3	46
10	36.7	35.8	52	31.8	31.6	56
11	37.2	_	69	29.5	_	71
12	28.9	_	75	27.9	_	77
13	36.8	_	65	31.1	_	69
14	34.3	_	71	32.8	_	73
15	33.8	_	72	30.8	_	78
16	32.8	_	56	29.4	_	59
17	36.2	_	58	33.0	_	56
18	38.1	_	64	32.8	_	62
19	32.8	_	59	31.3	_	57
20	34.9	_	57	33	_	55
21	33.6	_	54	32.3	_	55
22	35.8	_	44	32.1	_	49
23	36.6	_	46	33.4	_	49
24	36.7	_	45	32.4	_	46
25	35.1	_	49	31.6	_	53
26	34.8	_	43	31.5	_	48
27	35.2	_	68	32.1	_	68
28	38.1	_	63	32.7	_	65
29	36.1	_	55	32.1	_	57
Mean±Sem	34.86±**0.42**	33.01±**0.52**	49.23±**2.95**	31.47±**0.28**	30.31±**0.26**	49.97±**3.33**

PROJECTS DEVELOPMENT INSTITUTE (PRODA)

(FEDERAL MINISTRY OF SCIENCE AND TECHNOLOGY)

PRODA ROAD, EMENE INDUSTRIAL LAYOUT, P. M. B. 01609, ENUGU

Tel:

Fax:

Ref:

Date: 2nd April, 2015.

ANALYSIS RESULT:

SAMPLE DESCRIPTION: DIFFERENT MAGGOT SPECIES

SOURCE: C/O ABAJUE MADUAMAKA C, NNAMDI UNIVERSITY, AWKA, ANAMBRA STATE

ANALYSIS REQUIRED: ATOMIC ABSORPTION SPECTROPHOTOMETRY ANALYSIS OF ZINC AND PHOSPHORUS IN MAGGOT

SAMPLE IDENTIFICATION	PARAMETER (mg/g)	
	ZINC	PHOSPHORUS
D4	0.040	0.002
SPg D7	0.046	Not detected
PPg D7	0.058	Not detected
PD8	0.079	Not detected
D 10	0.084	Not detected
D 14	0.060	Not detected

Dr. (Mrs.) N. C. Onyemelukwe
Head MET