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Determination of the post-mortem interval (PMI) using insect succession patterns on BALB/c Mice (Mus musculus) cadavers in coastal Kenya

Lawrence Onyango Oswago[1]*, Celestine Makobe[1] & Joseph Baya Msanzu[2]
[1]Department of Medical Microbiology, Jomo Kenyatta University of Agriculture and Technology (JKUAT), Kenya
[2]Department of Pure and Applied Sciences, School of Medicine and Health Sciences, Technical University of Mombasa
https://orcid.org/0009-0004-3686-4107
***Corresponding author's email:** *lawrenceoswago@gmail.com*

ABSTRACT
Accurate estimation of the post-mortem interval (PMI) remains a major challenge in forensic investigations, particularly in regions lacking baseline entomological data. This study investigated decomposition dynamics and arthropod succession patterns on BALB/c mice cadavers in indoor and outdoor environments at the Technical University of Mombasa, Kenya. The aim was to estimate was to estimate post-mortem interval (PMI), in coastal Kenya, a region that lacks baseline entomological data, even though a local newspaper reported extrajudicial killings that resulted in bodies being dumped in the mangrove forests in the coastal regions or near rivers and lakes on the western parts of the country. A prospective longitudinal experimental design was employed using 272g laboratory-bred BALB/c mice exposed under controlled conditions where decomposition stages, insect colonization, and species succession were recorded daily at intervals of 6 hours and statistical analysis was done at 95% significance level with p-value of 0.05. The ten families were found at both indoor and outdoor sites. Major arthropod families observed included Calliphoridae, Sarcophagidae, Muscidae, Phoridae, Formicidae, Dermestidae and Cleridae, with incidental taxa such as spiders and millipedes. Necrophagous flies dominated early stages, whereas dermestid beetles were more prominent during the late stages of decomposition. The observed successional patterns followed predictable temporal waves corresponding to the different stages of decomposition. These findings provided baseline forensic entomology data for coastal Kenya and demonstrated that insect succession on BALB/c mice can be used to estimate PMI in medico-legal investigations while also highlighting the importance of localized entomological data in improving the accuracy and reliability of postmortem interval (PMI) estimation in tropical environments.

KEYWORDS: post-mortem interval, forensic entomology, insect succession, decomposition, tropical climate, Kenya

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INTRODUCTION

Immediately after death, a sequence of physical, biochemical and ecological changes occur in the animal body, providing critical indicators for estimating the post-mortem interval (PMI). Post-mortem interval is globally used in criminal law cases where forensic entomological investigators are often employed in the investigation of suspicious deaths because insects can provide vital information on when, where and how death occurred (Mashaly and Al-Mekhlafi, 2016). Forensic entomology is an area of entomology, which applies insects and other arthropods in criminal investigations. The insects found in a decomposing vertebrate corpse or carrion can be used to estimate the time of death such as time interval between death and corpse discovery, movement of the corpse, manner and cause of death and association of suspects at the death scene (Sukontason *et al*., 2007). Determining the PMI of both animal and human remains in veterinary and medico-legal contexts is often of paramount importance, particularly in respect to establishment of mode of death, time of death and place of death (Cattaneo, 2007). Forensic entomology, or the application of arthropod science to legal matters, has steadily increased its prominence within the forensic sciences (Brundage and Byrd, 2016).

Forensic entomology involves the application of insect biology to legal investigations, particularly the estimation of PMI through developmental stages and succession of necrophagous insects (Catts & Goff, 1992). Blowflies (Calliphoridae) and flesh flies (Sarcophagidae) are typically among the first colonizers of the carrion, depositing eggs or larvae within hours after death (Amendt *et al.,* 2007). Subsequent waves of insects exploit the resource in a predictable sequence corresponding to decomposition stages. Despite the recognized importance of forensic entomology, there remains a critical gap in localized data for tropical coastal environments, particularly warm and humid environments such as Coastal Kenya and many more tropical environments (Okiwelu *et al.,* 2008). Environmental conditions such as tropical climate, humidity, and local insect fauna significantly influence decomposition rates and colonization patterns. Consequently, the findings from temperate regions cannot be directly applied to tropical Africa.

Animal models are commonly used in forensic studies to simulate human decomposition. BALB/c mice are advantageous due to their availability, low cost, manageable size, and phylogenetic proximity to humans within the Euarchontoglires clade. Establishing baseline insect succession data using this model can enhance PMI estimation in regions lacking human decomposition studies. This study aimed to determine PMI using insect succession on BALB/c mice cadavers exposed in indoor and outdoor environments in coastal Kenya. Specific objectives were to document decomposition stages, identify arthropod species involved, and characterize succession patterns.

MATERIALS AND METHODS

Study Site

The study was conducted at the Technical University of Mombasa, located in Tudor, Mombasa County, Kenya with a Latitude of - 4.055 and Longitude of 39.664. The region experiences a tropical coastal climate with average temperatures ranging between 28 °C and 30 °C during the study period. This study was conducted in February- March, 2020.

Study Design

A prospective longitudinal experimental design was employed. There were 6 replications for each site. Decomposition was monitored continuously after every 6 hours from the time of death until advanced decay.

Experimental Animals

Laboratory-bred BALB/c mice (*Mus musculus*), approximately 12 weeks old and weighing about 272 g, were obtained from the animals rearing facility of Department of Biochemistry of Kenyatta University

which is an accredited breeding facility. The strain was selected due to its genetic uniformity and widespread use in biomedical research. The study animals were transported using ventilated, escape-proof, disinfected transport boxes lined with absorbent bedding. The mice were observed for injury or infection upon arrival and transferred to clean cages with food and water immediately. They were acclimatized for 48 hours before the actual experiment.

Inclusion criteria

Laboratory-bred BALB/c mice (*Mus musculus*), Adult mice (≥ 12 weeks old), Body weight approximately 250–300 g, Clinically healthy with no visible diseases and No external injuries or deformities

Exclusion Criteria

Sick, weak or malnourished mice, Juvenile or aged mice outside target age range, Body weight outside 250–300 g range, Presence of wounds, infections, or congenital abnormalities, Evidence of parasitic infestation and Carcasses contaminated during handling

Sample Size Determination

Resource equation method was used to determine the sample size for BALB/c mice used in this study (Charan and Kantharia, 2013).

Where: **E** = Error degrees of freedom,

N = Total number of animals and **T** = Number of groups

E=18–3=15

E=18÷3 = 6 mice per group

Sampling Technique

A total of 100 BALB/c mice were received from the breeding facility for sampling. The BALB/c mice used in the experiment were selected using simple random sampling (SRS). For every ten males, four were randomly picked and for every ten females, six were randomly picked.

Experimental Setup

Two exposure environments were established: indoor laboratory environment and outdoor enclosures with wire mesh cage measuring 3 m × 5 m. Each environment had 6 replicates. Carcasses were protected from vertebrate scavengers while allowing insect access.

Euthanasia and Carcass Placement

Mice were humanely euthanized by surgical incision (neck slitting) using a sterile scalpel. This was done after deeply anesthetizing the mice to achieve a painless death (National Institutes of Health, 2013). Carcasses were immediately placed at designated sites.

Monitoring of Decomposition

Gross post-mortem changes were documented daily through visual inspection and photography. Observations included: body temperature changes, rigor mortis, livor mortis, bloating, tissue breakdown and skeletonization.

Arthropod Collection and Identification

Arthropods were collected daily from carcasses and surrounding areas using forceps and sweep nets. Forceps were used to pick crawling and stinging arthropods whereas sweep nets were used for flying insects. Both adults and immature stages were recorded where present. The specime were stored in ethanol filled collection jars and taken to the laboratory. Specimens were identified to family level using standard entomological identification keys.

Data Analysis

The data was stored in laboratory notebook and entered in Microsoft Excel. The data was then analyzed using Statistical Package for the Social Sciences (SPSS) and Microsoft Excel. Qualitative descriptive data was presented as narratives while quantitative for different treatments were subjected to student t-test and summarized in the form of means. Statistical significance was examined at 5% significance level. The data was presented in form of tables and graphs where necessary.

Ethical Considerations

Ethical approval for this study was sought from Technical University of Mombasa- Institutional Scientific and Ethics Review Committee (ISREC).

RESULTS

Gross changes in decomposition of BALB/c mice

Five decomposition stages were observed and recorded: fresh stage, bloated, active decay, advanced decay and skeletonization (Figures 1-5). Fresh stage (Figure 1) began immediately after slitting the throats of the BALB/c mice and displaying them to the various sites. During the day, the cadavers became warm and at night the bodies cooled to match the ambient temperature of the surrounding, a condition known as *algor mortis*. By the next day, the cadavers became so rigid and difficult to relax its muscles after about 12 hours, a condition termed *rigor mortis*. The fresh stage went up to Day 2.

Figure 1: BALB/c mouse cadaver during fresh stage in Outdoor Site

Bloating (Figure 2) was observed from Day 3 when the cadavers began to putrefy and an odorous smell was produced that attracted a huge number of Dipterans on both the outdoor and indoor scenes. From day 5, the cadavers at the outdoor site were extremely ruptured (Figure 3) leading to loss of tissues, shading off the skin and fur while the cadavers on the indoor scenes were slightly ruptured. This marked the beginning of active decay. There were different developmental stages of maggots in both sites. On the outdoor sites, third instar maggots were observed migrating away from the carcass.

Figure 2: BALB/c mouse cadaver during bloated stage of decomposition. A mass of blow flies was attracted to the odorous smell.

Figure 3: BALB/c mouse cadaver during active decay stage

On the 6th day, a single spider and a checkered beetle was observed in the outdoor site accompanied by few blow flies, flesh flies and ants, whereas few blow flies and house flies were observed in the indoor sites. On the 7th day, few blow flies and flesh flies were observed in both sites. This marked the end of advanced decay. On the 8th day, both the cadavers were showing signs of dryness thus marking post-decay and skeletonization.

Figure 4: BALB/c mouse cadaver during Advanced Decay

Figure 5: Skeleton of the BALB/c mouse cadaver during Post Decay

Arthropod Diversity

Adult stages of arthropods were captured for identification from both the outdoor scenes and indoor scenes throughout the study, based on morphological characteristics. Three insect orders were identified: Diptera (Calliphoridae, Sarcophagidae, Phoridae and Muscina), Coleoptera (Cleridae and Dermestidae) and Hymenoptera (Camponotus ants, small carpenter ants, ponerine ants and crickets) were captured and identified.

Four dipteran families (Sarcophagidae, Calliphoridae, Muscina and Phoridae), Hymenoptera and two Coleopteran families (Cleridae and Dermestidae) were observed on the BALB/c mice cadavers at the outdoor scenes whereas three dipterans (Calliphoridae, Sarcophagidae and Muscina), Hymenoptera and one family of Coleoptera (Dermestidae) were recorded at the indoor scenes. All these species arrived at different times of the decomposition of the BALB/c cadavers.

Table 1: Arthropod groups observed in 8 days of decomposition period

Day after death	Order	Arthropod groups observed
1	Diptera	Calliphoridae , Sarcophagidae and Formicidae
2	Diptera	Calliphoridae, Sarcophagidae, Formicidae, and *Musca domestica*
3	Diptera	Calliphoridae, Sarcophagidae, Formicidae, and *Musca domestica*
4	Diptera	Calliphoridae, Sarcophagidae
5	Diptera	Calliphoridae, Sarcophagidae
6	Diptera & Coleoptera	Calliphoridae, Sarcophagidae, Formicidae and Cleridae
7	Diptera & Coleoptera	Calliphoridae, Sarcophagidae and Cleridae
8	Diptera & Coleoptera	Calliphoridae, Sarcophagidae, Cleridae and Dermestidae.

Arthropod Occurrence during Decomposition

The mean number of Arthropods groups collected in 10 days is shown in Table 2 and Figure 6-7 below. There were more blow flies and ants in day 1, blow flies, ants and house flies in day 2, more blow flies

and flesh flies in day 3, more blow flies and flesh flies in day 4, more ants and blow flies in day 5, more ants, flesh flies and checkered beetles in day 6, more blow flies and flesh flies in day 7, more flesh flies and checkered beetles in day 8, more house flies and skin beetles in day 9 and more skin beetles and spiders in day 10.

Table 2: Daily mean for each Arthropod species collected from both outdoor and indoor sites

Arthropod group	Mean number of Arthropods in each day										Overall mean
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	
Flesh flies	4.00	5.67	2.67	2.83	0.67	0.83	0.33	1.17	0.67	0.00	1.88
Blow flies	7.50	25.17	4.49	3.33	1.50	1.17	0.50	0.50	0.00	0.00	4.42
House flies	0.00	7.50	0.00	0.00	0.00	0.00	0.00	0.00	1.83	0.00	0.93
Ants	8.50	8.00	0.00	5.84	4.83	2.50	3.16	0.00	0.00	0.00	3.28
Skin beetles	0.00	0.00	0.67	0.00	0.00	0.00	0.00	0.00	0.33	1.00	0.20
Checkered beetles	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.67	0.00	0.00	0.10
Spiders	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.02
Millipedes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.03

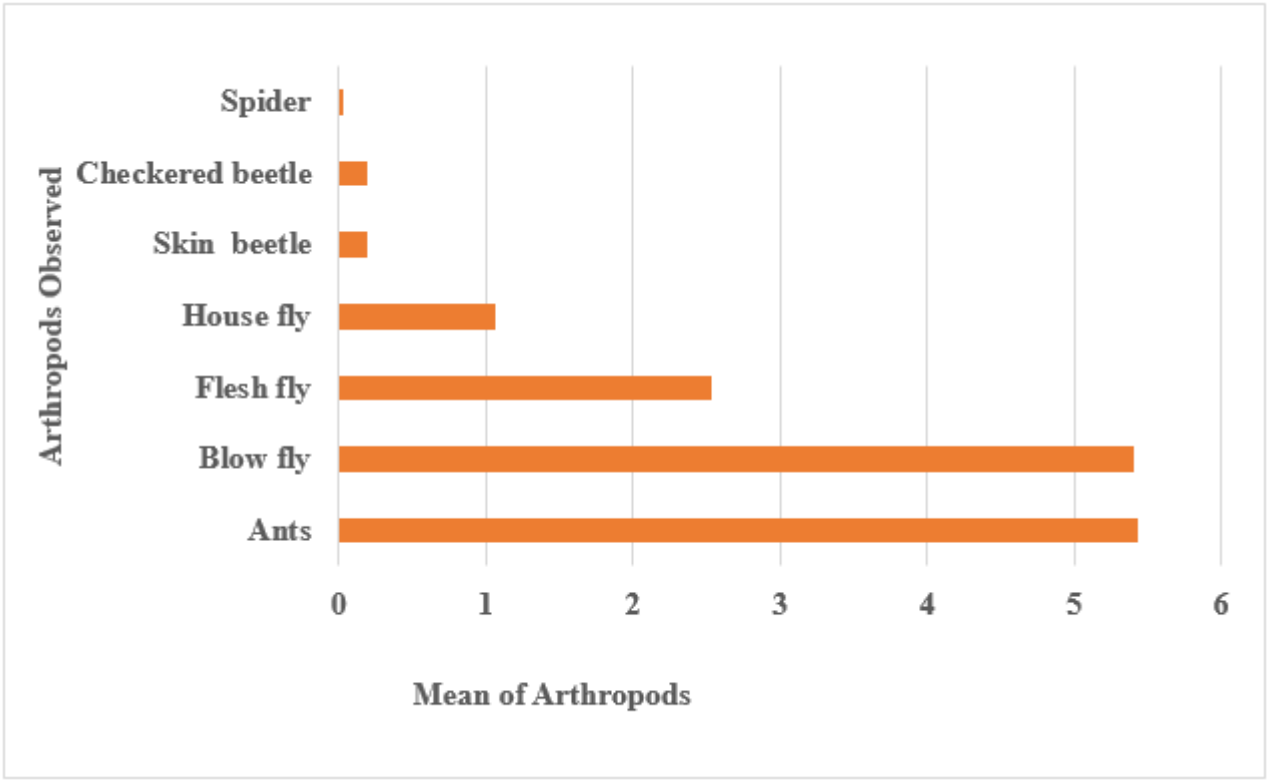

Figure 6: Outdoor mean number of arthropods observed daily

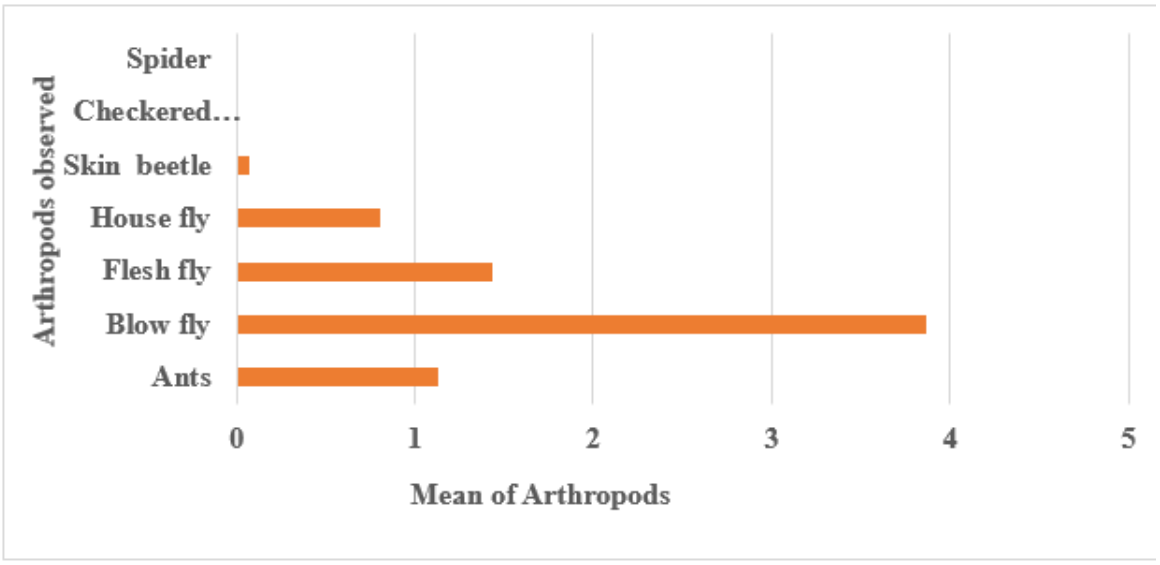

Figure 7: Indoor mean number of Arthropods observed daily

Arthropod succession patterns during decomposition of BALB/c mice

Dipterans arrived immediately after the BALB/c mice were killed and exposed at the different outdoor sites. It took 3 hours for the dipterans to arrive and colonize the BALB/c mice cadavers at the indoor scenes. The species observed on the first day of exposure were: house flies (*Musca domestica*), flesh flies and blow fly's species were observed from the fresh stage to the decay stage. From the second day to the fourth day, the number of Calliphoridae, Sarcophagidae and *Muscina* increased due to the odor smell produced by the BALB/c mice cadavers during the bloating and decay stages. Phoridae was observed on the fourth day during the decay stage. Blow flies (Calliphoridae) provided the largest number of flies on both the outdoor and indoor scenes followed by house flies (*Musca domestica*) and flesh flies (Sarcophagidae).

On the third and fourth day of decomposition, maggots of different developmental stages were seen feeding on the cadavers in the indoor scenes whereas maggots on the outdoor scene were seen migrating to the soil for pupation. On the sixth day of decomposition, few blow flies and flesh flies were seen accompanied by a spider which is an incidental species at the outdoor scene. Two families of Coleopteran (beetles) were seen during this study. They included Cleridae (checkered beetle) and Dermestidae (skin beetles). The checkered beetles were observed on the eighth day in the BALB/c mice cadavers at the outdoor scene while skin beetles were seen on the eighth day and tenth day for the indoor scene and outdoor scene respectively. Hymenoptera such as camponotus ants, small carpenter ants and ponerine ants were observed from the fresh stage to the skeletonization. Incidental species such as millipedes were observed near the skeleton of the BALB/c carcass on the tenth day.

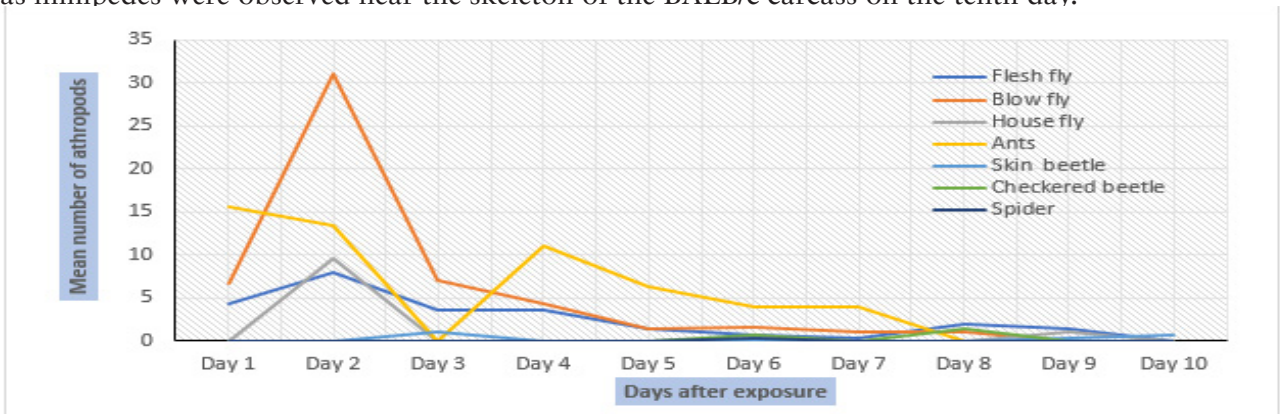

Figure 8: Indoor mean comparison of Arthropods (Insect succession pattern)

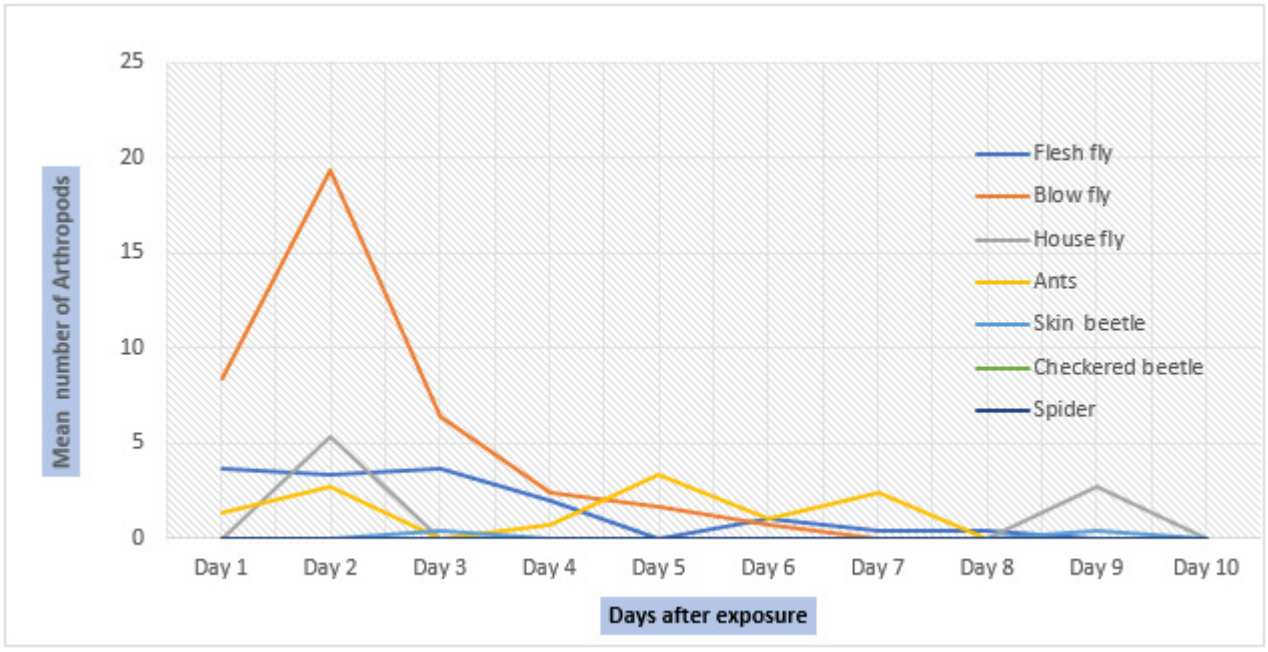

Figure 9: Outdoor mean comparison of Arthropods (Insect succession pattern)

DISCUSSIONS

The decomposition stages observed in this study corresponded closely with classical models of vertebrate carrion breakdown described in forensic literature. Rapid colonization by blowflies and flesh flies confirmed their role as primary indicators for early PMI estimation. These insects possess acute olfactory receptors enabling detection of decomposition odors shortly after death as illustrated by Amendt *et al.*, 2007. Maggot masses observed during active decay significantly accelerated tissue breakdown through both feeding activity and metabolic heat production. Elevated temperatures within larval aggregations can exceed ambient temperature, further accelerating decomposition as consistently observed in the study by Byrd and Castner, 2010.

Environmental exposure played a crucial role. Outdoor carcasses decomposed faster due to increased insect access, solar radiation and fluctuating humidity. Indoor carcasses showed delayed colonization, illustrating how restricted access can lead to PMI underestimation if not considered during forensic analysis. Ants were abundant throughout the study and likely influenced decomposition dynamics by preying on fly eggs and larvae. Their continuous presence supports previous findings that omnivorous insects can alter successional patterns by reducing necrophagous populations. This was consistent with the findings of Okiwelu *et al.*, 2008. Late-stage colonization by dermestid beetles is consistent with their ecological preference for dry tissues. Their presence signals advanced decomposition and can aid PMI estimation when soft tissues are absent.

The predictable succession sequence observed demonstrates the reliability of entomological evidence in PMI determination. However, geographic specificity remains critical. Tropical environments exhibit faster decomposition rates than temperate regions, emphasizing the need for localized reference data. The use of BALB/c mice proved effective as a small-animal model. Their small body size allowed rapid observation of complete decomposition cycles, making them suitable for experimental forensic studies where larger animal models may be impractical. These findings were similar to those of Abajue *et al.*, 2015 in Northern Nigeria.

CONCLUSIONS

This study demonstrated that insect succession on BALB/c mice cadavers followed predictable patterns corresponding to decomposition stages in coastal Kenya. Necrophagous flies dominated early stages, while beetles characterized later phases. Environmental conditions influenced colonization timing and decomposition rate.

Recommendations

Establish regional entomological reference databases across different Kenyan ecological zones to help in solving extrajudicial killings which remain unsolved due to in ability to determine time since death of the victims. Moreover, conduct studies using larger animal models to approximate human decomposition.

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Conflict of Interest: The author declares no conflict of interest.

Funding: This research received no external funding.

Institutional Review Board Statement

This study involved the use of laboratory animals (BALB/c mice) and was conducted in accordance with accepted guidelines for animal handling and experimental procedures.

Data Availability Statement:

Data supporting the findings of this study are contained within the article.

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