

**DETERMINING THE FLY AND MAGGOT  
POPULATION BASED ON DIFFERENT TYPES  
OF ANIMAL MEAT**

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# **DETERMINING THE FLY AND MAGGOT POPULATION BASED ON DIFFERENT TYPES OF ANIMAL MEAT**

**by**

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## LIST OF SYMBOLS

|     |                |
|-----|----------------|
| °C  | Degree Celcius |
| %   | Percentage     |
| hrs | hours          |
| g   | grams          |

## LIST OF ABBREVIATIONS

|                       |                                                |
|-----------------------|------------------------------------------------|
| PMI                   | Post-mortem interval                           |
| <i>C. Megacephala</i> | <i>Chrysomya Megacephala</i>                   |
| <i>C. Rufifacies</i>  | <i>Chrysomya Rufifacies</i>                    |
| <i>M. Scalaris</i>    | <i>Megaselia Scalaris</i>                      |
| <i>D. Cornuta</i>     | <i>Dohrniphora Cornuta</i>                     |
| <i>Sarcophaga sp.</i> | <i>Sarcophaga species</i>                      |
| UPMS                  | Science Lab Management Unit                    |
| 1st                   | First                                          |
| 2nd                   | Second                                         |
| 3rd                   | Third                                          |
| $H_0$                 | <i>Null Hypothesis</i>                         |
| $H_A$                 | <i>Alternative Hypothesis</i>                  |
| VOCs                  | Volatile Organic Compounds                     |
| MET                   | Official Department Of Meteorology<br>Malaysia |
| a.m.                  | <i>Ante meridiem</i> (before noon)             |
| p.m.                  | <i>Post meridiem</i> (after noon)              |

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# MENGENALPASTI POPULASI LALAT DAN ULAT BERDASARKAN JENIS DAGING HAIWAN YANG BERBEZA

## ABSTRAK

Serangga nekrofagus memainkan peranan yang sangat penting dalam entomologi forensik, khususnya dalam penganggaran selang masa selepas kematian (post-mortem interval, PMI). Secara khusus, lalat nekrofagus menjadi fokus utama dalam kajian berkenaan kerana ia merupakan organisma terawal yang menjajah bangkai dan mayat yang mereput. Kajian ini bertujuan untuk menilai perbezaan antara spesies lalat nekrofagus yang dapat dijumpai atas pelbagai jenis daging haiwan, iaitu daging khinzir, kambing dan lembu. Eksperimen ini dijalankan sebanyak dua kitaran, iaitu semasa musim hujan dan musim kering, bagi menilai pengaruh faktor persekitaran termasuk hujan, suhu ambien dan kelembapan pada masa yang sama.

Penemuan utama kajian ini ialah perbezaan antara keutamaan pemakanan yang jelas ditunjukkan oleh *Megaselia Scalaris* (Diptera: Phoridae) dan *Sarcophaga* sp. (Diptera: Sarcophagidae) yang didapati hanya memilih daging lembu, manakala *Chrysomya Megacephala* memakan semua jenis daging haiwan yang dikaji dan *Chrysomya Rufifacies* hanya memakan daging khinzir dan daging lembu. Selain itu, faktor persekitaran memainkan peranan penting dalam mempengaruhi kadar pereputan dan aktiviti serangga, yang menyebabkan penangguhan dalam proses pereputan dan oviposisi oleh lalat nekrofagus. Walau bagaimanapun, dapatan kajian ini hanya terhad kepada pengenalanpastian perbezaan antara keutamaan pemakanan dalam kalangan lalat nekrofagus, manakala punca asas kepada perbezaan tersebut masih belum dapat ditentukan.

# **DETERMINING THE FLY AND MAGGOT POPULATION BASED ON DIFFERENT TYPES OF ANIMAL MEAT**

## **ABSTRACT**

Necrophagous insects play a crucial role in forensic entomology, particularly in the estimation of post-mortem interval (PMI). Specifically, necrophagous flies have been the focus of relevant studies as they are the earliest colonizer of decomposing carcasses and cadavers. This study focuses on investigating the differences between the necrophagous fly species associated with different types of animal meat, which are pork, lamb, and beef. The experiment was conducted twice during rainy and non-rainy seasons to simultaneously examine the influence of environmental factors, including rainfall, ambient temperature, and humidity. A key finding was the distinct feeding preferences exhibited by *Megaselia Scalaris* (Diptera: Phoridae) and *Sarcophaga sp.* (Diptera: Sarcophagidae) exclusively towards beef, while *Chrysomya Megacephala* were observed to feed on all types of animal meats studied, and *Chrysomya Rufifacies* feeds on both pork and beef. Additionally, environmental factors played a key role in influencing the decomposition rate and the insect activity, which leads to delayed decomposition and the oviposition of necrophagous flies. However, the findings of this study were limited to identifying the difference in feeding preferences among necrophagous flies, while the underlying causes were yet to be determined.

## **CHAPTER 1 : INTRODUCTION**

### **1.1 Background of Study**

In forensic science, after a body is found, there are certain aspects that need to be examined that are significant for the investigation. Therefore, post-mortem examinations are important, as this is where professional knowledge and skills are utilized to collect and derive as much information as possible from the condition, appearance, wounds or injuries, and presence of foreign substances. This information is especially useful in aiding the investigation of the cause of death, manner of death, and time of death, which are significant for case investigation.

Regarding time since death, also known as post-mortem interval (PMI), there are already several well-researched and well-established methods that have been recorded to have reliable results. For example, in forensic anthropology, dental tissue was successfully utilized for radiocarbon dating to estimate the PMI (Milani et al., 2025). PMI determination through dating techniques is usually more applicable for cases where only skeletal remains were found, and the most well-recognized method would be carbon-14 radioisotope measurement (Franceschetti et al., 2023). Through post-mortem examination, it is also possible to estimate the PMI based on the temperature, condition, decomposition stage, and the stomach content of the body. Software has been invented that analyzes parameters like the body temp and solute concentrations in the vitreous humor in order to determine the PMI in a more efficient and practical way (Scendoni et al., 2025).

## **1.2 Problem Statement**

The focus of the paper will be forensic entomology, which centers around the insects that are attracted to the dead body during the decomposition process. In forensic entomology, flies and beetles are the most commonly mentioned insect species as they are constantly found to be colonizing the decomposing body. These insects are as widely known as necrophagous, which are known to oviposit and feed on the decaying matter. Research has been heavily done on the necrophagous insects due to their usefulness in contributing hints regarding certain aspects of the cadaver. These include indicating the presence of drugs or toxic substances, whether the cadaver has been moved, and wounds or injuries on the body (Hou et al., 2025). Most importantly, necrophagous insects have been extremely significant in determining the PMI of the body. It is certain that there are fixed colonizing and ecological succession patterns of different species of insects, and the life stage of the insect corresponds to the stage of decomposition of the decomposing body (Lutz et al., 2021).

Undeniably, different species or families of necrophagous insects have their own unique timing and preference towards the condition of the body when they are colonizing. This raises the question of when different species will choose to colonize and how they will choose. Research regarding the species identification has always been a hot topic in forensic entomology, and the necrophagous insect found certainly varies between different geographical locations, the condition of the body, such as buried, wrapped, or in water, climate, and temperature (Mayer & Vasconcelos, 2013; Hodecek & Jakubec, 2022). Feeding preference of necrophagous insects have been found to be varied among different types of

meat as well, where certain insect species would be found in abundance on specific types of meat as compared to other meats (Vilte et al., 2020; Qubaiová et al., 2022)

Specifically, this project aims to address the research gap of the relationship between the fly and maggot populations and different types of animal meat. Although there were a few relevant studies that have been done, there was no specific research, especially in Malaysia, that specifically uses animal meat of beef, lamb, and pork. This research aims to investigate how different types of animal meat affect the feeding preferences of various types of fly species. By looking into the timings of oviposition, maggot population, and the fly species that infest the animal meat, we believe that this study would provide a fresh perspective and significant contributions in filling the knowledge gap mentioned.

### **1.3 Objectives**

#### **1.3.1 General Objective**

To understand the association between the fly and maggot population and the type of animal meat.

#### **1.3.2 Specific Objectives**

1. To assess the effect of different types of animal meat on the feeding preference of necrophagous flies.
2. To compare the timing and rate of oviposition of various fly species on different types of animal meat.

3. To investigate the effect of environmental factors like rainfall, temperature, and humidity on the rate of decomposition, insect succession, and the rate of flies' development.

#### **1.4 Research Questions**

1. Do different types of animal meat lead to different species of flies that infest the meat?
2. Does feeding preferences of fly species relate to the timing and rate of oviposition on animal meat?
3. How does the factor of rainfall, temperature, and humidity affect the rate of decomposition, insect succession, and the rate of flies' development?

#### **1.5 Research Hypothesis**

*Null hypothesis ( $H_0$ ):*

There is no significant association between the fly and maggot populations and the types of animal meat.

*Alternative hypothesis ( $H_A$ ):*

There is a significant association between flies and maggot populations and types of animal meat.

#### **1.6 Significance of the Study**

This study provides a new insight into highlighting a probable association between types of animals and their effect on the feeding preferences of various fly species. In forensic entomology, necrophagous flies hold an incredibly high evidential value in PMI estimation

due to their consistent and predictable colonization pattern and life cycle. Existing studies have briefly proposed regarding the variation in feeding behavior and preferences towards different types of animal meat, but were based in other countries with different climate conditions and flies' population, which makes it less suitable to be applied in Malaysia.

By investigating the fly species that colonize different types of animal meat, it was expected that there would be variation in species and abundance of maggot and flies found across different types of animal meat. This allows a new possibility to be explored, which focuses on the reason behind the feeding preferences of flies towards different types of animal meats. Additionally, the findings of this study may contribute to an improved interpretation of forensic entomological investigation, especially in PMI estimation. By recognizing the differences in feeding preferences among various species of flies indigenous to Malaysia, an increase in credibility and reliability of forensic entomological evidence can be enhanced.

## **CHAPTER 2 : LITERATURE REVIEW**

### **2.1 Decomposition**

Decomposition is the natural process that takes place once an organism is dead, where the corpse or carcass starts to break down, and the process will be affected by various extrinsic and intrinsic factors. Decomposition has been the biggest part of forensic science, especially in estimating the postmortem interval (PMI). Other than estimating the time of death, it is also crucial in reconstructing possible events, identifying remains, which aids in forensic investigation, as well as the legal procedures.

Throughout the years, researchers have been focusing on studying the decomposition pattern as well as the influencing factors. For instance, a study that was conducted in 2024 that focused on the influencing factors contributing to the variation in the decaying process of a body highlighted that based on the current findings, the most commonly studied factors are microbes and insects in comparison to volatile organic compounds (VOCs), pre-appearance interval (PAI), oviposition, drugs and toxins, and there were no direct correlations in between those factors. This study also emphasized the knowledge gap as well as the importance of collaborative efforts in enhancing the research on the decomposition study (McIntyre et al., 2024).

Another study reviewed the decomposition process and how the extrinsic variables change the post-mortem process as well as the interval, especially from the perspective of forensic taphonomy. Decomposition was described as a process consisting of two stages, which are autolysis, that refer to the cellular degradation process of a cell caused by hydrolytic enzyme

and then followed by the degradation of tissue by microorganism activity, which is called putrefaction. The post-mortem changes that were addressed in the review include mummification and saponification, which could preserve the corpse and interfere with the estimation of PMI. This study implies that there are still gaps in knowledge regarding the correlations between the known variables that contribute to the decomposition process of a corpse (Pasca & Ulasan, 2014).

An article that was published in 2018 highlighted the significant advances in the research of decomposition, specifically in the discipline of forensic anthropology, such as the primary extrinsic factors, detection of clandestine remains, and estimation of PMI in forensic anthropology. For example, the invention of decomposition facilities allowed researchers to conduct more comprehensive longitudinal studies, which could greatly improve the outcome and the understanding of the primary extrinsic factors. An important recommendation addressed in the article was to focus on the collection of more standardized data, as well as proposing the idea of conducting more interdisciplinary or transdisciplinary studies (Wescott, 2018).

## **2.2 Stages of Decompositions**

Decomposition mainly consists of five stages, which are fresh, bloated, active decay, advanced decay, and skeletonization. These stages are determined by the conditions of the corpse and the postmortem changes. The determination of the stage of decomposition aids in the estimation of PMI, as each of the stages typically lasts for a period of time before entering the next stage, and the rate of decomposition is almost the same under the same

circumstances. In Malaysia, which is a tropical climate country with year-round hot and humid weather with occasional rain, the first four stages of the decomposition process normally last for a month before entering the skeletonization. The characterization of the stages of decomposition provides a framework for estimating the time of death and holds a certain investigative value.

### **2.2.1 Fresh**

As explained above, the first stage will be autolysis, which is also known as the fresh stage. The fresh stage normally lasts for about two days before entering the bloated stage. After death, the body will undergo several changes, such as algor mortis, livor mortis, rigor mortis, and autolysis. Algor mortis, which is the cooling of the dead body after death until in equilibrium with the environmental temperature (Christensen, Passalacqua and Bartelink, 2014). Livor mortis is caused by the lack of blood circulation as the heart stops working. This results in the settling of blood due to gravity, where discoloration at the lower part of the body can be observed. Livor mortis is usually seen at least one hour after death and could last for 9-12 hours before the condition is constant, where there are no more discolorations observed, even after applying pressure (Lee Goff, 2009).

At the same time, the lack of necessary biochemical reactions causes the decreases of ATP production, which is needed in muscle contraction, resulting in the locking of myosin and actin filaments. Thus, the muscle becomes inflexible and stiff (Madea and Kernbach-Wighton, 2013). Autolysis is also when the cell breaks down by hydrolysis enzymes start due to the absence of a metabolism process, which causes the cell's acidity to increase, and

eventually the cell walls burst to release enzymes (The Stages of Human Decomposition, 2025). Soon after death, the decomposition of the dead body will attract the flies, especially blowflies, where they will lay eggs on the body, specifically at the openings like genitals and anal openings, eyes, mouth, ears, nose, or any open wounds.

### **2.2.2 Bloating**

Around the third day, as the cells continue to break down, gases produced from the decomposition start to accumulate inside the body, which results in the bloating of the dead body as well as the discoloration to a greenish color. The most obvious observation of a body in the bloating stage is that the body is approximately doubled in size due to the gas accumulation. The gas produced, which is hydrogen sulfide, will diffuse into blood vessels and react with hemoglobin to form a complex with green pigments, and the greenish color is especially more obvious in the region of livor mortis (Lee Goff, 2009). At this stage, is where the putrefaction starts, which includes stage bloating until skeletonization. A strong, unpleasant, and long-lasting odor started to emanate from the body.

The digestion of hydrolytic enzymes continues at the junction between the layer of epidermis and dermis, which causes the epidermis layer to be shed and become a layer of *stratum corneum* that is constantly being removed and replaced with the underlying layer. This is what we call skin slippage. Other than that, marbling is also a phenomenon that is observed during the bloated stage. Similar to the discoloration of livor mortis, the activity of anaerobic bacteria, which then results in the production of sulf-haemoglobin, with the green pigments invading into the subcutaneous blood vessels. Over time, the discoloration can range from

purple-greenish to brownish-grey. The pattern seen from the discolored vessel on the body resembles the mosaic flooring appearance and hence the name of the marbling (Pasca & Ulasan, 2014; Lee Goff, 2009).

### **2.2.3 Active decay**

Active decay is where the body fluids start to leak through orifices. The soft tissue, muscle, and organs start to liquify, which contributes to the great mass loss of the body. The degradation rate increases dramatically at this stage, as there are more open or ruptured areas available for the insect activity, as well as for the scavengers, which will consume the soft tissues. This stage typically begins on about the fifth day and lasts for about a week. When most of the soft tissue is exhausted, the degradation rate decreases to a very slow rate, which brings it to the advanced decay stage (The Stages of Human Decomposition, 2025).

### **2.2.4 Advanced decay**

Advanced decay is basically the same as active decay, but at a much slower rate. The remaining soft tissue will continue to liquefy until there are only hard bones and dry tissue left, such as hair, bones, and cartilage, which require more time to degrade due to their resistance to decomposition and lack of moisture. At this stage, there is only a faint smell of the pungent odor, and the insect activity decreases drastically. The major indication of this stage is the gradual replacement of the blowflies (Dipteran species) by the beetles (Coleoptera species). This is due to the preference of the species towards the habitat and feeding conditions. Since most of the soft tissue is gone, the remaining dry environments are not suitable for blowflies, while beetles feed on cartilage and bones, and they are better adapted

to the dry environment. Hence, the Dipteran species migrate to pupate while the Coleoptera species takes over the remains. This is called insect succession (Pasca & Ulasan, 2014; Lee Goff, 2009).

Depending on the environments surrounding the body, conditions such as mummification and saponification may happen, which will prolong the advanced decay stage. In a dry and extreme temperature condition, the inhibition of microbial and insect activities causes the desiccated soft tissue to have a leathery appearance. This condition could last for several weeks. Saponification refers to when corpse wax forms on the outer layer of the body, which will delay the decay process. This is due to the warm, damp, and alkaline environment, usually in soil or water, which allows the hydrolysis of body fat by the anaerobic bacteria that results in a greasy wax which will harden over time (Stolze, 2014).

#### **2.2.5 Skeletonization**

When most of the soft tissue is gone, leaving a large portion of the skeleton exposed, the decomposition process enters the skeletonization stage. Skeletonization usually starts after a month and could last for years. Over time, exposed skeletons may experience changes like bleaching, weathering, or cracking. The rate of decomposition of a skeleton is largely based on the environmental setting, where it would take much longer for a skeleton on fertile soil to decompose than on neutral soil or sand (Carpenter, 2024).

## **2.3 Influencing factors of decomposition**

The rate of decomposition can be affected by certain factors such as temperature, location, humidity, physical barrier, burial setting, etc. These factors not only affect the decomposition process of the carrion, but it also influences the colonization and development of the insect infestation on the body, and these factors should be considered in the calculation of PMI. It is crucial to understand the correlation between these factors and the effect on the decomposition process of carrion and colonization, as well as the development of the insects.

### **2.3.1 Temperature**

Temperature is considered one of the most important influencing factors, as it has a direct influence on the colonization and survival of the bacteria and insects. Microbial activity, enzymatic activity, and insect infestation can be inhibited at extreme temperatures, and the carrion can be preserved for a longer time. For instance, in countries with seasonal weather, there will be a great difference between the decomposition rate of carrion during different seasons due to the different temperatures. In polar temperatures, human remains can be found in intact condition even after thousands of years, as the extremely cold temperature makes it impossible for bacteria to survive, and there is zero microbial activity that could lead to decomposition (Campobasso et al., 2001).

### **2.3.2 Toxins and Drugs**

The presence of toxins and drugs in the dead body, as well as the surrounding environment, could bring differences in the colonization pattern of insects and microbial activity during the decomposition of the carrion. The presence of drugs at a certain concentration level could alter the cellular reaction of the decomposition, which could lead to inaccurate PMI estimation and cause of death identification if a drug detection test is not taken. Cases of intoxication or drug overdose leading to death are not rare nowadays, and there are also cases of environmental pollution. These situations can be the source of drugs and toxins detected during post-mortem toxicology analysis. A few studies discovered the potential of certain drugs as biomarkers for PMI estimation. Fortunately, recent advancements in the method and instruments for toxicology analysis have brought improvements in the accuracy and reliability of drug and toxin detection to increase the investigative and evidential value (Li et al., 2024).

### **2.3.3 Insect Access**

Insect infestation is the nature of a decomposition process, where it enhances the decomposition rate by feeding on the carrion. Restriction on the insect's access to the carrion will affect the decomposition rate, as the decomposition process depends solely on microbial activity by bacteria and digestion by hydrolytic enzymes. This results in a much slower decomposition rate as compared to normally exposed carrion. Circumstances like buried carrion and the presence of clothing or physical barriers like wrapping or cement could limit the insect access and restrict the insect infestation process.

Buried carrion was found to have a much slower decomposition rate than exposed carrion lying on the ground. The difference in the decomposition can be up to four times, where buried carrion took 42 days to reach skeletonization, while ground control carrion only took 10 days. The same goes for the presence of clothing or a physical barrier on the cadaver. When there is clothing covering the cadaver, it seems to prolong the wet decay stage and allow larger maggot masses to move freely on the cadaver. Wrapped animal carcasses had a delayed arrival of insects, while mummification and saponification were observed on cemented animal carcasses even after a few months. An experiment was done in which the decomposition rate of an animal carcass with total exclusion of insects seems to be twice as slow as that of the control carcass (Soon, 2017).

## **2.4 Forensic Entomology**

In forensic investigation, insects that are found along with the dead body are greatly significant as they hold certain forensic investigative value, especially in estimating PMI. To estimate the PMI using insects, one must possess enough knowledge regarding the life cycle of the insect found on the dead body or carcass. In general, an insect's life cycle consists of four stages: egg, larva, pupa, and adult. By identifying which stage of the life cycle the insect is currently at, we can estimate the time of death.

According to a literature review done by Matuszewski (2021), it was revealed that there are still gaps and errors in the current execution of how the authorities collect the insect evidence from the death scene. He proposed that it should be revised and improved, where the personnel who are assigned to collect the insect evidence should have a certain background and knowledge regarding forensic entomology, which allows them to choose the most competent and suitable insect evidence to avoid issues like a lack of diversity and evidential value. When collecting and choosing the insect evidence, the decomposition appearance should be observed to have a basic judgment of which stage of decomposition the cadaver or carcass is at, as different stages of decomposition correspond to different life cycle stages of the insect. For instance, if the cadaver or carcass is at a fresh stage, the target insect evidence should be the eggs or larvae of flies (Matuszewski, 2021).

Other than PMI estimation, insect evidence has shown other significant investigative aid, such as showing if the body has been moved to a second site or any disruption by animal or human after. With sufficient knowledge regarding the ecology and behavior of insects,

especially blowflies and beetles, the insect evidence may give clues to the possible events, such as premortem injuries, exposure to drugs or toxins, as well as aiding in the reconstruction of possible events leading to death (Bhuyan et al., 2025).

## **2.5 Necrophagous Insects**

As mentioned above, soon after death, insects will start getting attracted to the dead body and oviposition on the body. Based on studies and research made, there are two major species that are involved in the decomposition process, which are Dipteran species and Coleoptera species. Rather than inhabiting the dead body at the same time, it was observed that insect succession occurs where the Diptera species will first take over the dead body and then be replaced by the Coleoptera species at different stages of decomposition due to the differences in the preferences towards breeding and feeding environment.

Based on a study conducted in Malaysia with an indoor setting, firstly, there were three scuttle flies (blowflies) observed, which are *Megaselia Scalaris*, *Megaselia Spiracularis* and *Dohrniphora Cornuta*. Findings of this study address the potential of *D. Cornuta* to be forensically significant for indoor cases. Additionally, *M. Scalaris* was found to be the dominant fly species to be the earliest to reach the carcass, and its high occurrence in indoor cases makes it highly reliable as the sole indication for PMI estimation in the absence of other Sarcophagus species (Syamsa et al., 2015). Another study conducted in Selangor, Malaysia, which aims to study the scuttle flies that can be observed with plastic-sealed carcass, also emphasized the predominant scuttle fly species for indoor cases, which is *M. Scalaris* that proved its forensic significance (Raja M. Zuha et al., 2017).

Depending on the environmental setting of the dead body, different types of fly species can be found. Another example, a case report regarding a dipteran species found on a dead body in high-rises. There were two dipteran species found in all three cases reviewed in the case report: *Megaselia Scalaris* and *Synthesiomyia Nudiseta*. Both of the fly species were also commonly found in indoor cases. This study proves that altitudes may affect the type of fly species that can be found on a dead body. This study highlights another influencing variable that should be studied more (Syamsa et al., 2015).

Diptera and Coleoptera are the orders of insects that are commonly encountered and studied in forensic entomology. The Diptera order refers to flies, while the Coleoptera are beetles. A study done in 1997 proved that both diptera and coleoptera are forensically significant in relation to a dead body or carcass. Based on the result, a few species of Calliphoridae and Sarcophagidae from diptera order and a few species from coleoptera order were observed. For example, *Chrysomya Albiceps*, *Chrysomya Megacephala*, *Chrysomya Putoria*, *Dermestes Maculatus*, *Dermestes Peruvianus* (Dermestidae) (Souza and Linhares, 1997).

### **2.5.1 Diptera**

Diptera, also known as true flies, are one of the most forensically significant insects in forensic entomology due to their ability to detect and reach a cadaver or carcass almost immediately after death. Thus, by deducting the time of colonization mathematically based on the life cycle stage of flies and the lower and upper thresholds for the survival of the flies, the PMI can be estimated (Brundage, 2020). Diptera flies, whether larvae, eggs, or adult

flies, all of them have distinctive morphological characteristics that help confirm if they are dipteran flies as well as differentiating between different species of the family.

Based on the studies done on dipteran flies that took place in Malaysia, it can be seen that the most commonly encountered fly species that have been proven to be forensically significant are: phoridae (scuttle flies), sarcophagidae (flesh flies), and calliphoridae (blow flies) (Tan et al., 2010; Raja M. Zuha et al., 2017; Jones et al., 2024). Different species of flies are usually found in different environmental settings, such as indoors, outdoors, and high-rises. Hence, the species of flies collected may indicate whether the body has moved to a different place.

Other than morphological identification of fly species, researchers propose DNA identification using a part of the flesh flies (flesh flies) like the legs. The data from the genes analyzed from the flies collected can be matched to the databases to better identify and differentiate the dipteran flies into separate clades for better understanding and accurate analysis. This allows the overcoming of the issue where morphological identification is a challenge due to similar characteristics or a lack of research done on the less encountered species. In the findings, there are no overlapping values obtained from the DNA analysis of each species or clades, which suggests that the genetic marker of each species is extremely specific (Tan et al., 2010).

Firstly, after the death of a human or animal, the decomposition process of the dead body will attract the insect, specifically the dipteran female true flies, to come and lay eggs (oviposition) on the orifices and open wounds of the dead body. This happens on the fresh

stage of the decomposition process. The eggs will then hatch into first instar larvae during the fresh stage, and bloating and then develop until the third instar while feeding on the soft tissue of the dead body. During the development process of larvae, the maggot will move and spread across the whole dead body to feed on the soft tissue, where the rate of decay will increase rapidly, causing the release of strong, unpleasant odors that attract more dipteran and even coleoptera species. This corresponds to the active decay and advanced decay of the decomposition process of carrion. During the transition from advanced decay to skeletonization of the carrion, as most of the soft tissue has been digested and the larvae are ready to pupate, insect succession will occur where the larvae of dipteran species will be replaced by coleopteran beetles. The larvae of dipteran species migrate into soil to grow into adult flies. To deduct the PMI, the time of colonization should be calculated using a mathematical formula with the unit of DD (degree days) or DH (degree hours) (Brundage, 2020; Matuszewski, 2021).

#### 2.5.1.1 Calliphoridae (Blow Flies)

Blow flies are the most generalized species of flies in forensic entomology due to their abundance, and it is the earliest insect species to colonize decomposing meat. Its predictable and stable colonization patterns are useful in estimating the PMI as well as contributing to its evidential value. Adult blow flies are commonly named as blue bottle and green bottle flies due to their greenish and blueish metallic appearance that makes it differ from other species (Merritt et al., 2009). In most of the research done, the morphological characteristics of oral sclerite and spinose bands of third instar maggot were always the central focus in

differentiating between different species of blow flies (Szpila et al., 2019; Abass & Ali, 2024).

In another article, cephalopharyngeal skeletons of third instar maggot are useful for species identification. The third instar was chosen as the cephalopharyngeal skeleton is the most well-developed, and different parts of it varied slightly between different species of blow flies, making it useful for life stage identification as well. It also suggests that the parastomal bars have a different length ratio when compared to the hypopharyngeal sclerite for different species, and it is absent in *C. Rufifacies*. The length of the ventral cornue of *C. Rufifacies* is nearly half the sclerotized portion of its dorsal cornue, while for other species like *C. Megacephala*, *Cochliomyia Macellaria*, and *Lucilia Lucigerens*, their ventral cornue is half or the entire dorsal cornue (Barrett, Yuan and Garraway, 2022).

Unlike larvae, only limited research has been done on adult blow flies that are forensically relevant. One of the articles emphasized that the wing shape of adult flies is reliable for species identification, especially for *Chrysomya spp.*, where it shows a more distinctive characteristic difference than other species (Sontigun et al., 2017). This finding is also supported by another study that utilizes morphometric analysis of the wing shape of the adult flies, but the study focuses on the *Lucilia spp.* (Limsopatham et al., 2022). Although the species identification based on geometric landmarks on the wings is not 100% accurate, it is reliable and inexpensive as compared to methods using DNA barcoding.

### 2.5.1.2 Sarcophagidae (Flesh Flies)

Sarcophagidae, also known as flesh flies, are one of the necrophagous insects. Its forensic importance was since they are one of the colonizers of decomposing carcasses. Adult flesh flies have a gray thorax with black stripes and a hairy appearance, with a body size much larger than that of Calliphoridae flies, which makes it much more recognizable among various species of flies (Merritt et al., 2009). As studies suggest, different types of species of flesh flies are distributed among different countries. For example, *Sarcophaga Ruficornis* (Fabricius), *Sarcophaga Taenionota*, and *Sarcophaga Albiceps* are commonly found in Malaysia (Ren et al., 2018).

As adult blow flies, a study proposed findings of using wing shape as a morphological characteristic for species identification based on the landmarks. In contrast, this method does not have a successful rate as high as Calliphoridae due to its high similarity of characteristics, which reduces the accuracy of differentiation (Szpila et al., 2022). Another article has stated the same finding as well, in which species identification using wing shape is challenging and is not as reliable. This article suggested an alternative that utilizes the distinctive characteristic of the male genitalia of adult flies. Male genitalia were chosen due to insufficient significant differences of female genitalia between different species of Sarcophagidae. A challenge of this method is that male adult flies are much less commonly found than female flies (Reis et al., 2025).

For third instar larvae, instead of the cephalopharyngeal skeleton, an article published by Szpila, Richet and Pape (2015) has concluded that the anterior spiracle has a higher taxonomic value in species identification of Sarcophagidae. To be exact, the significant characteristics are the shape of the spiracle, the number of lobes, and their arrangement. The distribution of spines, i.e., spinose bands and integumental structure, can be significant as well, as it is easy to observe and capture by using standard stereomicroscopes. This provides the character with a high taxonomic value.

#### 2.5.1.3 Phoridae (Scuttle Flies)

Phoridae, also known as scuttle flies, have a much smaller size compared to Calliphoridae and Sarcophagidae. Its humpbacked appearance in adult flies is where it gets its nickname, humpbacked flies (Merritt et al., 2009). The most common species would be *Megaselia Scalaris*, especially in Malaysia. Phoridae are commonly recorded in cases of buried or indoor cadavers (Han et al., 2025; R. A. Syamsa et al., 2017). Phoridae are forensically significant for indoor settings and concealed bodies, as they have the advantage of being small and able to approach smaller openings, while the arrival of larger flies, like blow flies, is restricted (Reibe and Madea, 2010).

Due to its small size and characteristic with high taxonomic value, species identification of scuttle flies based on morphological characteristics has been a great challenge, and there are only a few articles that have researched this specific issue, while most of them resort to alternatives such as DNA sequencing or DNA barcoding (Namaki-Khameneh et al., 2021).

An article had focused on implicating species identification of Phoridae using wings' vein landmarking on adult flies, but the accuracy was only 52%-67% (Brown & Wong, 2020).

A study done in Malaysia regarding confined animal carcasses has discovered 6 species of Phoridae. The article stated that the species identification of Phoridae relies heavily on the male flies, which means that the identification of female flies can only be done by linking to the male of the same species. Bristles and tergites of the adult flies are highly significant for the identification of *Spiniphora spp.* (Raja M. Zuha et al., 2017).

## **2.6 Oviposition**

Oviposition is forensically important as it is the key to the PMI estimation, as it marks the time of colonization of the insect on the cadaver. Regarding oviposition of flies, which is the process of laying eggs, different modes of oviposition should be noted, which are oviparity, viviparity, and ovoviviparity.

For instance, Calliphoridae flies, and Phoridae are usually oviparous, where they lay eggs on the meat which will then hatch into maggot. This aids in estimating PMI more accurately (Yang et al., 2021). Viviparity is where the adult flies lay larvae directly onto the cadaver instead of laying eggs. Most commonly found Sarcophagidae species in Malaysia are usually viviparous, which is why they are usually only observed after they become adult flies (Merritt et al., 2009). On the other hand, there are several other species of Sarcophagidae flies that are ovoviviparous, which indicates that eggs are present and will hatch in the female flies before being released as first-instar larvae (Yang et al., 2021).

There are influencing factors that lead to preferences of different fly species towards different conditions or locations of the meat. For example, a study that focused on fly species under the family Sarcophagidae in different locations on the meat carcass. The finding suggests that Sarcophagidae flies prefer to oviposit on orifices on the head, like the mouth, eyes, ears, and nose, rather than other body parts, and never on wounds. This conclusion only applied to outdoor cases, as the research also suggests that eggs can be found on the wound, but only under overcrowded conditions (Rivers, Ferrone and Mulroy, 2023).

Nocturnal oviposition should also be considered in studies related to the oviposition behavior of necrophagous flies. Studies have suggested that necrophagous flies will oviposit at night as well as during the daytime, but at a much lower rate. Most relevant necrophagous flies would be Calliphorid, which have been the focus of the majority of related studies (Kirkpatrick and Olson, 2007; Carneiro et al., 2021). A study proposed that the likelihood of nocturnal oviposition of flies has to be supported by the condition where the environmental temperatures are appropriately high (Amendt, Zehner and Reckel, 2008).

Undeniably, temperatures are one of the significant influencing factors of oviposition behavior of necrophagous flies, where studies have stated that oviposition will only occur when the environmental temperature is within the range of the upper and lower thresholds (Ody, Bulling and Barnes, 2017; Hans, LeBouthillier and VanLaerhoven, 2019). For instance, research done on *C. Rufifacies* has discovered that they can oviposit within the environmental temperature range 22.5 to 42.5 °C, but the eggs are not viable when the temperature exceeds 37.5°C (Rusch et al., 2025).