POST-MORTEM INTERVAL COMPARISON IN DIFFERENT MEAT CONDITIONS

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LIST OF SYMBOLS

º C	degree celsius
g	gram
%	percent
ml	milliliter
m	meter

LIST OF ABBREVIATIONS

MSF	Makmal Sains Forensik
PMI	Post Mortem Interval
PPSK	Pusat Pengajian Sains Kesihatan
UPMS	Unit Pengurusan Makmal Sains
USM	Universiti Sains Malaysia
VOC	Volatile Organic Compound
KOH	Potassium Hydroxide

POST-MORTEM INTERVAL COMPARISON

IN DIFFERENT MEAT CONDITION

ABSTRACT

Lack of data in different post-mortem interval estimation conditions limit the application of entomological evidence in crime investigation. Therefore, this study investigates the decomposition stages, insect succession patterns, complete life cycle of fly and larva consumption in fresh meat, frozen meat, dry fresh and dry frozen meat. There are 5 decomposition stages for the fresh and frozen meat : fresh, fresh decay, advance decay, post decay and dry whereas 3 decomposition stages for the dry fresh and dry frozen meat were dry, dry decay and remains. The duration for the fly to complete their life cycle is within 13 days at an average ambient temperature of 28.80 ºC with relative humidity of 76.50%. Six necrophagous fly species, beetles with several ants were observed in both decomposed fresh meat and frozen meat but only ants were observed in both dry meat. For larva consumption in each of the meat conditions, the average meat consumed per larvae was 0.68 g in fresh meat, 0.67 g in frozen meat, 0.54 g in dry fresh meat and 0.47 g in dry frozen meat. These decomposition stages, insect successions patterns, the complete life cycle of the fly and larva consumption information deserve consideration as it provides valuable baseline data for post-mortem interval estimation related to different conditions of the dead body found in shaded areas in any forensic entomology cases specifically in Kelantan.

PERBANDINGAN ANGGARAN MASA KEMATIAN

PADA DAGING YANG BERBEZA KEADAAN

ABSTRAK

Kekurangan data bagi bedah siasat anggaran masa kematian dalam keadaan yang berbeza mengehadkan penggunaan bukti entomologi dalam penyiasatan jenayah. Oleh itu, kajian ini menyiasat peringkat penguraian, corak penggantian serangga, kitaran hayat lengkap lalat dan corak pemakanan larva dalam daging segar, daging beku, daging sejuk beku kering dan daging segar kering. Terdapat 5 peringkat penguraian bagi daging segar dan beku: reput segar, reput segar, pereputan awal, reput pasca dan kering manakala 3 peringkat penguraian bagi daging segar kering dan daging beku kering adalah kering, reput kering dan sisa. Tempoh lalat untuk melengkapkan kitaran hayatnya adalah dalam tempoh 13 hari pada suhu purata 28.80 ºC dengan kelembapan relatif 76.50%. Ada enam spesies lalat nekrofag, kumbang dengan semut telah direkodkan berada pada daging segar yang reput dan daging beku tetapi hanya semut yang direkodkan pada kedua-dua daging kering. Bagi corak pemakanan larva dalam setiap keadaan daging, purata daging yang dimakan oleh setiap larva ialah 0.68 g dalam daging segar, 0.67 g dalam daging beku, 0.54 g dalam daging segar kering dan 0.47 g dalam daging beku kering. Peringkat penguraian, corak penggantian serangga, kitaran hayat lengkap lalat dan maklumat corak pemakanan larva wajar dipertimbangkan kerana ia akan memberikan data asas bagi anggaran selang masa kematian dalam setiap bedah siasat yang berkaitan dengan keadaan mayat yang berbeza yang ditemui di kawasan tersembunyi atau tertutup dalam mana-mana kes berkaitan dengan entomologi forensik khususnya di Kelantan.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Forensic entomology is a branch of forensic science that emerged and used in forensic investigation as crime increases in today's society. It is the name generally used for any examination of the insect that cooperates with the legal issue and is sometimes known as 'forensic medical entomology' or 'medico-criminal entomology' (Singh et al., 2019). Forensic entomology nowadays is accepted as one of the developing fields and serve as an alternative to support medical examination and determination in death analysis and investigation.

In general, forensic entomology is used to determine the time between death and the discovery of the dead body (Raju, 2019). There are many applications of forensic entomology in a forensic science investigation but the most common one is the estimation of time since death or known as post mortem interval (PMI). Therefore, forensic entomology is a lot more synonym to PMI estimation. The time estimation is based on the entomological species deposited on a dead body and the complete life cycle of the species. Byrd (2019) stated that the age of the insect collected from the dead body can aid in determining the PMI and the time estimation is usually based on the period of insect activity in the remain.

1.2 Problem Statement

Post-mortem interval is known important in determining the death interval of a dead body. The determination of the time range is generally estimated by the deposition of the entomological specimen on the body. The potential of forensic entomology reveals that it is useful in assisting crime investigation by providing information on where, when and how under certain conditions a crime was being committed as emphasized by Faran et al., (2018).

It has been reported that there are several research on the estimation of PMI in Malaysia had been done before but many previous studies only highlight certain baseline data in certain cases (Syamsa et. al, 2015). However, the PMI of different meat media conditions which are equally important for forensic entomological investigation in Malaysia remain unreported. In light of this, the fact that some criminal hides dead body that they had killed in a shaded area, disposed in a freezer and burned the body in the oven or direct fire was existed in certain cases. Not only reported in overseas, on 3rd of July 2018, Malaysia was shocked with the unusual act of brutality and inhumanity as a 5 months old baby was found at his babysitter's house in a freezer (Harian Metro, 2018). In another case reported by The Star (2022), an unidentified woman with a burned trauma was found on May 31st by the riverbank of Sungai Kuala Kangsar. Considering the situation, a specific study focus on post-mortem interval estimation and entomological data in different meat conditions might be useful and might benefit any entomological-related case in any forensic investigation. Apart from that, basic knowledge of larva consumption on different feeding sources in this study also will be beneficial for further research related to the fly larva.

1.3 Objective

1.3.1 General Objective

To investigate the PMI estimation in fresh meat, frozen meat, dry fresh meat and dry frozen meat.

1.3.2 Specific Objective

1. To identify the insect succession patterns and the decomposition stages in 4 different meat conditions
2. To determine the complete life cycle of the forensically important fly species in 4 different meat conditions
3. To compare the larvae consumption in 4 different meat conditions

1.4 Significance of Study

Post-mortem interval is vital as it could assist in the identification of the deceased and aid in the estimation of the time of death when the body was found after 72 hours (Gennard, 2007). In Malaysia, most of the post-mortem interval estimation was based only on one condition of animal model. Therefore, the determination of the PMI in this study could bring a basic fundamental result for an early death time interval estimation in 4 different meat conditions. The larvae consumption analysis could provide basic and fundamental information on the feeding behaviour of the fly as the selected prominent necrophagous species. This study also would be valuable as a reference baseline data as well as the data collected will be useful for discriminating the condition of the dead bodies found in any cases related to the forensic entomological investigation in Malaysia.

CHAPTER 2

LITERATURE REVIEW

2.1 Forensic Entomology

Forensic entomology is the study of the insect species that deal with the morphology, classification, distribution and physiology of the insect in a crime investigation (Meena et al., 2020). The time elapsed since death is an important matter in any forensic analysis such as the information might aid in both victim and criminal identification when the death has no witness.

2.2 Forensic Entomology to Solve Crime Scene Investigation

The estimation of the PMI from forensic entomology is the most valuable when any other parameter including medical examination no longer could be used. Autopsy interval basically was used to determine the time of death. However, this procedure is restricted and only applicable to the first 48 to 72 hours after death (Campobasso et. al, 2001). Therefore, the PMI using developmental data of certain species of insect and the arthropod community composition analysis was used as a calculation and the estimation of the time of interval (Bala, 2018).

2.2.1 Decomposition Stages and Insect Succession Patterns

Decomposition stages play a very important role in determining the time elapsed since death or known as the estimation of PMI. Details observation and understanding of the appearance of the decomposition process might help in the investigation process.

As the evidence from the decomposition stages and the insect succession is progressing and emerging across the globe, scientists and the court system nowadays used forensic entomology to assist in crime investigation, particularly to estimate the time interval in human death cases (Rajesh Singh et al., 2022).

It is also known that the information from insect succession is reliable to show the duration of decomposition. Recognition of the varying array of insects in a predictable chronological sequence would reveal the stage of decomposition and later on, the time elapsed since death (Sardar et al., 2021). The presence of the insects could tell how a person died by the existence of the wound or trauma on the body, the last place where the deceased had been seen, the location where the deceased had been killed or the location where the deceased was buried before being disposed to another place. A previous study in Malaysia by Syamsa et al. (2017) supports a combination of information and knowledge of the studies can enhance and assist investigation related to forensic entomology cases.

2.2.2 Necrophages Insects Involve in PMI Estimation

As soon as the death occurs, the decomposing process start and the insect which are attracted to the dead body will play an active role in the decomposition (Rana, 2020). Generally, there are four categories of insects that can be found on a dead body which are the necrophages such as beetle and flies, the omnivores such as wasps and ants, parasites and incidentals. Most of these insects are attracted to the decayed and decomposed tissue from the dead body and some of them use the corpse as new habitat.

In Malaysia, necrophagous flies are the forensically important species selected to estimate the minimum post-mortem interval. Although the most prominent species found were *Chrysomya rufifacies* and *Chrysomya megacephala,* there were also other species such as *Chrysomya* villeneuvi *Patton, Chrysomya nigripes Aubertin, Chrysomya bezziana Villeneuve, Chrysomya pinguis, Sarcophaga, Lucilia, Hemipyrellia ligurriens, Hemipyrellia, Ophyra spinigera, Synthesiomyia nudiseta* and *Eristalis* reported to be found in certain cases (Lee et al, 2004).

2.2.3 Experimental Model and Media Infestation

Post-mortem interval estimation mostly relies on specific cases reported for any crime scene investigation related to a dead body but the analysis would take longer as for any new cases, each determination had to undergo new examination and investigation. Therefore, an alternative analysis needs to be done so that the determination and the result could be concluded faster by utilizing the reference of previous data. Some of the researchers used human body as the experimental model and some from a real case (Lutz et. al, 2021). In Malaysia, some of the analysis also was based on real cases as being conducted by Syamsa et. al, (2017). However, it depends on the ethics and also stock and supply of the dead body to conduct specific investigation.

Nowadays, many researchers used animal to simulate a real dead body such as monkey, rabbit and meat as the experimental model as the sample was easily obtained and reliable to express the same results as a real body. In Malaysia, some researchers used rabbit (Nordin et. al, 2020; Mahat et. al, 2019) and some of them used beef meat (Nasir et. al, 2019) and buffalo meat (Ishak et.al, 2018).

2.3 Forensic Entomology in Malaysia

In Malaysia, forensic entomology field had gained interest and emerged as it become one of the methods to estimate minimum post-mortem interval. Table 2.1 shows the summary of forensic entomology cases and findings in Malaysia made by previous researchers in the last 5 years (2017).

Table 2.1 Summary of the published studies of forensic entomology cases and findings in Malaysia from 2017 to early 2022

No	Authors	Year	State	Area / Condition	Media infestation / experimental model	Necrophagous species
1	Syamsa et al	2022	Kuala Lumpur	Aquatic habitat	Human remains / corpses	Chrysomya megacephala, Chrysomya rufifacies, Eristalis spp
2	Nordin et al	2020	Sarawak	Primary forest	Rabbit carcasses	Hypopygiopsis violacea, Hypopygiopsis fumipennis, Hemipyrellia ligurriens, Hemipyrellia tagaliana, Chrysomya megacephala, Chrysomya rufifacies, Chrysomya villeneuvi, Chrysomya channi, Chrysomya pinguis, Chrysomya nigripes, Ophyra spinigera, Ophyra chalcogaster, unidentified Sarcophagidae
3	Mahat et al	2019	Johor	Decomposition site	Rabbit carcasses	Chrysomya megacephala, Chrysomya rufifacies, Hemipyrellia tagaliana, Ophyra chalcogaster, Ophyra spinigera and unidentified Sarcophagidae
4	Nasir et al	2019	Johor	Sunlit habitat	Decomposing beef substrates	Chrysomya megacephala
5	Ahmad & Omar	2018	Selangor	Simulation site	Rat, rabbit and macaque carcasses	Chrysomya megacephala, Chrysomya rufifacies
6	Ishak et al	2018	Pulau Pinang	Insectarium site	Minced buffalo meat	Lucia cuprina
7	Syamsa et al	2017	Kuala Lumpur	Indoor and outdoor	Human remains / corpses	Chrysomya megacephala, Chrysomya rufifacies, Sarcofagidae, Synthesiomya nudiseta, Megaselia scalaris, Lucia cuprina, Calliphorida nigripes, Eristalis spp, Hydrotaea spinigera

Previous research from 2017 until the present shows that there had been no recent study and findings related to forensic entomology in Kelantan. It is acknowledged that different region is restricted to their own climate and temperature regarding the differences in biogeoclimatic region. Therefore, this study would generally provide valuable data to assist in PMI estimation for any crime scene related to a dead body in Malaysia.

It is discovered that most of the previous investigation was done in an indoor and outdoor, from secluded to open area and also there are several cases in aquatic habitat. This study chose a secluded open area but limited to human exposure as it is the most prominent place to dispose of a dead body.

Previous researchers also focused on the same sample and animal models for their investigation as well as the real dead body found in any case reported. None of the previous study conducted under different conditions of the experimental model. This study however investigates decomposition stages and other basic essential data in different conditions of meat : fresh meat, frozen meat, dry fresh meat and dry frozen meat. This information could address the comparison and the difference between each of the meat condition used.

CHAPTER 3

METHODOLOGY

3.1 Locations and Study Sites

This study was carried out at the Health Campus of Universiti Sains Malaysia, Kubang Kerian, Kelantan at approximately 4.6 m above sea level, an eastern state of Peninsular Malaysia (606 “1” N, 102017 “5” E). Figure 3.1 illustrates the location where the study was conducted.

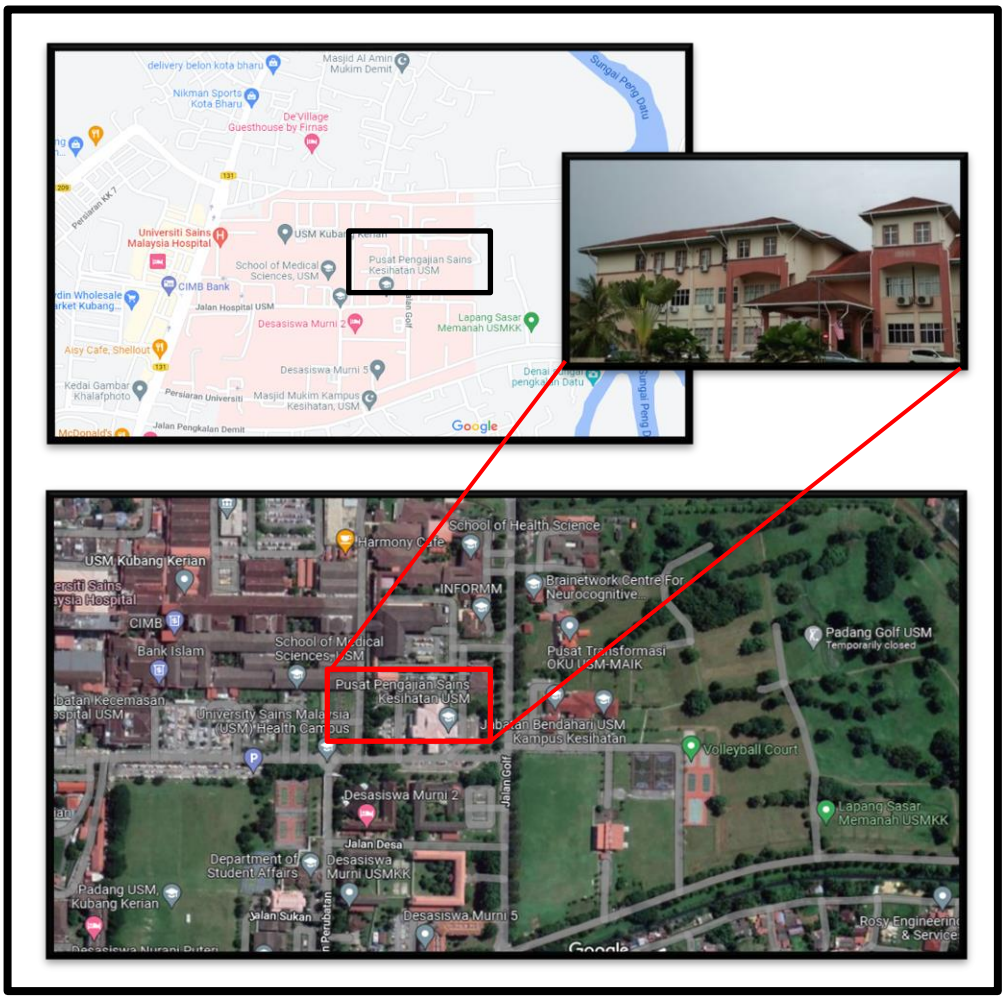

Figure 3.1 Maps showing School of Health Sciences, Universiti Sains Malaysia, Kubang Kerian, Kelantan, the location where the study was conducted (Courtesy of Google Maps 2022)

3.1.1 Selected Site of Study

Different locations around the School of Health Sciences Campus (PPSK), Universiti Sains Malaysia, Kubang Kerian, Kelantan were selected to study the comparison of the post-mortem interval estimation for 4 different conditions of meat. Each of the meat condition was placed at a different site of study and each of the chosen location was located approximately 50 m to 100 m from each other. Figure 3.2 shows the distance and the measurement of the study site from each other. Even though each location is located beside a parking lot, the area chosen has limited human exposure and not used as a walkaway to surrounding areas.

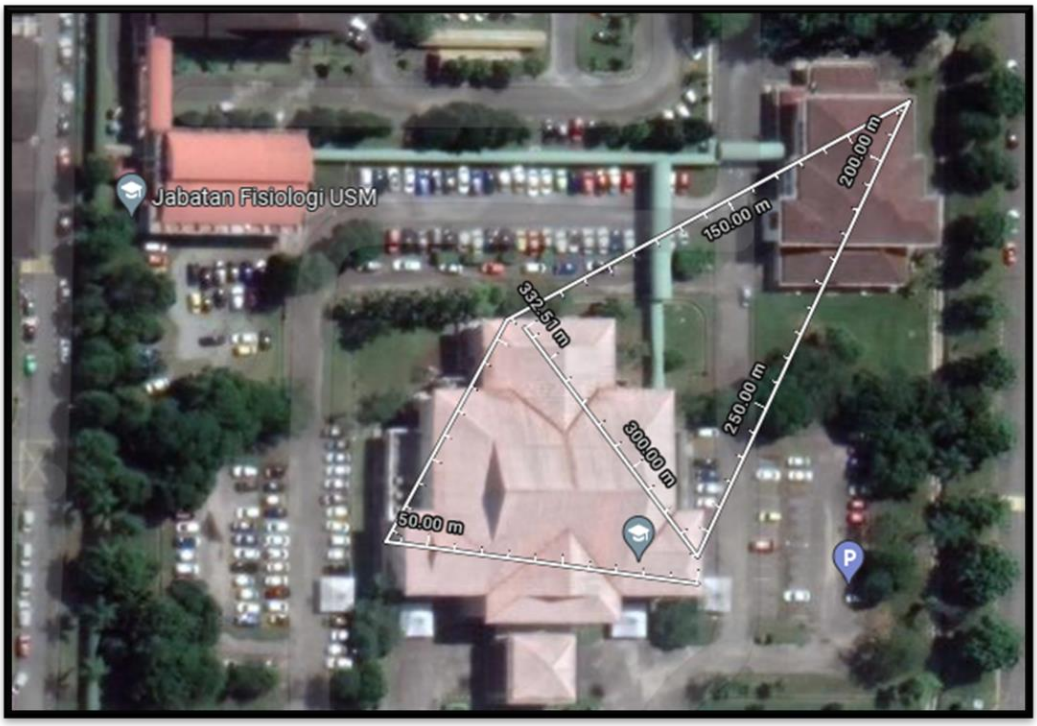

Figure 3.2 Maps showing distance and measurement of each location where the study was conducted (Courtesy of Google Maps 2022)

3.1.2 Location 1

Location 1 was located behind Dewan Kuliah Serbaguna near the School of Health Sciences (PPSK) buildings. Figure 3.3 shows Location 1.

Figure 3.3 Location 1 - behind Dewan Kuliah Serbaguna

3.1.3 Location 2

Location 2 was located behind Perpustakaan Hamdan Tahir at the PPSK parking lot. Figure 3.4 shows Location 2.

Figure 3.4 Location 2 – parking lot behind Perpustakaan Hamdan Tahir

3.1.4 Location 3

Location 3 was located at a drain near PPSK buildings. Figure 3.5 shows Location 3.

Figure 3.5 Location 3 – At a drain near PPSK buildings

3.1.5 Location 4

Figure 3.6 shows Location 4 which was located under the road to PPSK buildings.

Figure 3.6 Location 4 – near to the parking lot at PPSK buildings

3.2 Material

The frozen meat and the fresh meat were bought from Kubang Kerian market while dry meat will be prepared by heating the frozen and fresh meat in the Hot Air Oven Sterilizer overnight in the Food Laboratory, Universiti Sains Malaysia, School of Health Sciences, campus Kubang Kerian, Kelantan.

The chemicals such as chloroform, ethyl acetate and ethanol were obtained from Science Lab Management Unit (UPMS), School of Health Science, Universiti Sains Malaysia, Kubang Kerian, Kelantan. The chemicals, reagents, analytical apparatus, instruments and all the necessary equipment used in this research was shown in the table 3.1, 3.2 and 3.3 respectively.

Table 3.1 Chemicals and reagents used in this study

No	Chemicals and reagents	Manufacturer
1	Ethanol / Ethyl alcohol (30%, 50%, 70%, 80%, 90%)	Teraslab Saintifik, Malaysia
2	Chloroform	Merk & Co Inc, Germany
3	Ethyl Acetate	Merk & Co Inc, Germany
4	Potassium Hydroxide	Merk & Co Inc, Germany
5	Acetic Acid	Merk & Co Inc, Germany
6	Sodium Carbonate	Sigma Aldrich, Japan
7	Sodium Hydroxide	Merk & Co Inc, Germany
8	Bleaching Liquid	The Clorox Company, US

Table 3.2 Instrument used in this study

No	Instrument
1	Stereomicroscope (Leica MZ16)
2	Digital microscope camera (Leica MC170 HD)
3	Digital thermometer
4	Lab freezer / chiller
5	Hot Air Oven Sterilizer / Memmert Oven Laboratory
6	Digital balance / weighing balance
7	Vortex mixer (Erla EVM-6000)

Table 3.3 Apparatus used in this study

No	Apparatus
1	Latex gloves
2	Forceps
3	Face mask
4	Capped plastic vials / falcon tube
5	Zipped lock bag
6	Square plastic basket
7	Stone / bricks
8	Aluminium foil plate / rectangular disposable foil
9	Clear disposable plastic (blue / yellow)
10	Boards for field specimens
11	Wooden / metal spatula
12	Tissue
13	Rubber band
14	Plastic cups / container
15	Nylon cloth / some piece of mesh fabric
16	Petri dish
17	Measuring cylinder
18	Needles / applicator sticks
19	Beakers

3.3 Preparation for Different Conditions of Meat

Approximately 500 g of fresh meat, frozen meat, dry fresh and dry frozen meat were prepared and the experimental set up was done concurrently so that each of the meat media have the same environmental conditions.

Table 3.4 Preparation of Fresh, Frozen, Dry Fresh and Dry Frozen Meat

Meat condition	Quantity (g)	Preparation	Temperature (℃)
Fresh meat	500 g	-	-
Frozen meat	500 g	Thawed Overnight	Ambient Temperature
Dry fresh meat	500 g	Dry Overnight	105℃
Dry frozen meat	500 g	Thawed, Dry Overnight	105℃

Table 3.4 shows the preparation condition for each of the meat used in the study. All of the meat then was placed on a clear disposable plastic sheet.

3.3.1 Experimental Setup

The study was conducted concurrently for each of the meat conditions and the data was collected from 21st June 2022 to 21st August 2022. A basket was used at each location and a brick was placed on top and around the basket to avoid being eaten by any scavengers or predators. Figure 3.7 shows the experimental setup at each location.

Figure 3.7 Experimental setup at each of the locations with official notice for the study to be conducted (before stone were placed on top and around the basket)

3.4 Temperature and Meteorological Data Collection

The ambient temperature where each of the meat was placed was obtained using the digital thermometer approximately at chest height were recorded until the completion of the study. General weather data such as the relative humidity, as well as rainfall was obtained from the weather station office nearest to Universiti Sains Malaysia, Kubang Kerian, Kelantan combined with online weather forecast.

3.4.1 Observation and Collection of the Chosen Specimen

For a study using 4 meat conditions, each meat was being observed twice a day at 9 am and 3 pm for each location and was observed up to 62 days of the study. However, for the life cycle of fly species and larvae consumption study, each replicate was observed up to 15 days of the study. The amount of the entomological specimen populating the meat was noted and some sample of larvae fly was collected for further analysis.

Each of the stages of the fly larvae collected from eggs, 1st instar, 2nd instar and 3rd instar were preserved in 80% ethanol. Figure 3.8 shows the larva was preserved according to their species. Adult flies on the other hand were reared when it was almost in the pre-pupa stages. Each of the samples was taken for further analysis and for species identification.

Figure 3.8 Larvae specimens were collected and preserved in 80% ethanol
a) Larvae of Chrysomya rufifacies b) Larvae of Chrysomya megacephala

3.4.2 Larvae Morphology Acquisition

A larva specimen from preserved larvae in 80% ethanol was taken and placed on a flat and clean slide. Then the sub-posterior end of the larva was half cut using the scalpel blade. The half-cut larva then was immersed in a 10% potassium hydroxide (KOH) solution for 1 hour. The KOH solution was prepared by dissolving 10 g of KOH crystal with 100 mL of distilled water and stirred until the crystal completely dissolved. The solution was used to soften the the larva and avoid damaging the structure of the larva itself. All of the internal body content of the larva then was carefully removed by using soft forceps and a needle.

After removing all of the tissue content in the larva, the larva was transferred to 10% of acetic acid for 15 minutes. 10 mL of the acetic acid is diluted with distilled water in 100 mL volumetric flask. The larva then was soaked in ascending series of 30%, 50%, 70%, 90% and 100% ethanol for 30 minutes each. Each of the ascending series of ethanol was prepared accordingly from absolute ethanol combined with 70mL, 50mL, 30mL and 10 mL distilled water respectively. Figure 3.9 shows the ascending series of the ethanol used.

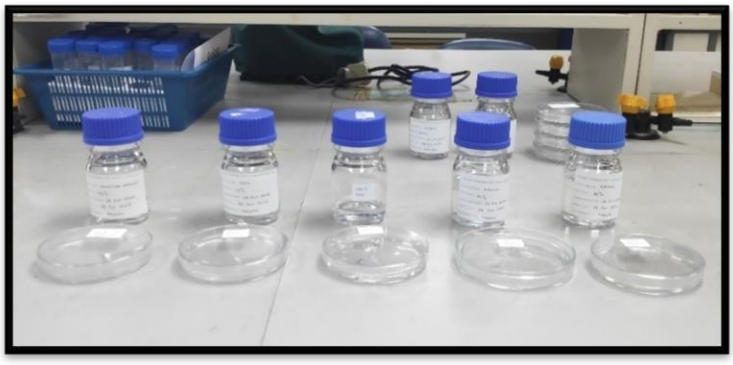

Figure 3.9 Larvae specimen soaked into the ascending series of ethanol

After 30 minutes soaked in ascending series of ethanol, the larva was placed on the glass slide and was ready to be examined under a microscope. Figure 3.10 shows the prepared larvae specimen ready for identification.

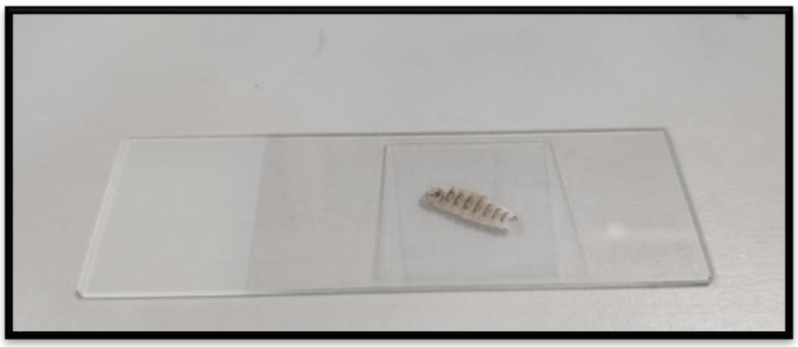

Figure 3.10 Larvae specimen on a glass slide

The method for clearing and washing the cephalopharyngeal skeleton was adopted from Sukontason et al, (2004) protocol. A larva is selected from the preserved 80% ethanol. A modified sharp blade and needle were used to dissect the anterior segment of the larva. The acquired cephalopharyngeal skeleton after being removed from the larva was placed in a test tube containing distilled water. Figure 3.11 shows the acquired cephalopharyngeal skeleton.

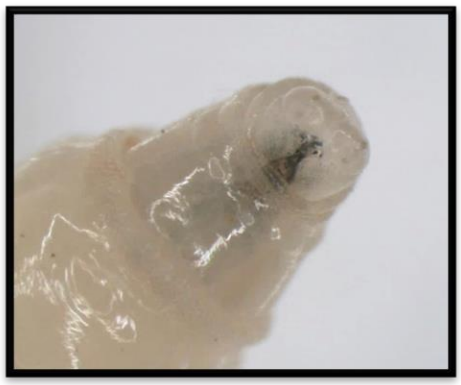

Figure 3.11 Acquired cephalopharyngeal skeleton

The cephalopharyngeal skeleton then is dissolved in a softening solution where the solution was prepared using 3.75 g sodium carbonate, 1 ml bleaching liquid, 29 ml distilled water and 20 ml of 15% sodium hydroxide and the cephalopharyngeal skeleton is incubated for 10 minutes in 80 °C water bath. The combined solution containing the cephalopharyngeal skeleton is then vortexed for 3 minutes to wash away the cuticle and the tissue attached to the skeleton.

After that, the cephalopharyngeal skeleton was taken out and distilled water is used to clean the remaining tissue at the cephalopharyngeal skeleton. The cephalopharyngeal skeleton then was transferred on the glass slide and was ready to be observed under the stereomicroscope.

3.4.3 Larvae Consumption

Approximately 15 g of meat is taken from 500 g of fresh, frozen, dry fresh and dry frozen meat respectively while 15 of 1st instar larvae were collected from another sample of meat used specifically for larvae sampling. Figure 3.12 shows the 1st instar larva collected at the study site.

Figure 3.12 1st instar larvae collected for larva consumption study

A petri dish was used for each replicate. 15 grams of the fresh, frozen, dry fresh and dry frozen meat was placed on the surface of each petri dish and 15 1st instar larvae was released on each of the meat.

The petri dish was enclosed with a basket covered by a mesh fabric to prevent other fly to oviposit on the meat. Figure 3.13 illustrate the experimental setup for larva consumption study.

Figure 3.13 Experimental setup for larva consumption study

The estimation of the larva consumption will be calculated based on [Eq.1]. The calculation was adopted from Amin (2019) and the step was used for each of the meat conditions in the experiment.

Larvae consumption calculation :

Meat consumed = Total meat weight – Remaining meat weight [Eq.1]

Meat consumed per larvae = Total meat consumed (g) / Total number of larvae

The weight of the remaining meat in each of the collected samples will be calculated separately and the average meat consumed will be calculated based on the data in each collected sample used in the experiment.

3.5 Rearing, Elimination and Preservation of the Specimen

A transparent container covered with nylon cloth was used to rear the specimen at the study sites from their pre-pupa stages. Half of the containers were filled with soils and elastic rubber bands were used to tighten the nylon cloth on the surface of the container to prevent the teneral to fly away when they emerged (Figure 3.14). Once the life cycle of the fly is complete, the adult fly was eliminated and preserved at the Forensic Laboratory (MSF), School of Health Sciences, USM.

Figure 3.14 Rearing fly species at the study site

Ethyl acetate was used for the elimination of the emerged teneral. A piece of tissue with a few drops of ethyl acetate was placed on top of the transparent container containing the emerged teneral and was left for 15 minutes. A large beaker was used to cover the whole container to aid in the elimination process. When all of the adult flies were laid down, all of the fly specimens were removed from the transparent container to several petri dishes before being dried overnight in the oven at 70 ºC to remove any water content from the fly specimen. Figure 3.15 demonstrate the experimental setup for the elimination process.

Figure 3.15 Experimental setup for the elimination process

CHAPTER 4

RESULTS

4.1 Decomposition Stages and Insect Succession Patterns

Decomposition stages were observed throughout all of the meat condition - fresh, frozen, dry fresh and dry frozen. Each day of the decomposition process was recorded as well as the ambient temperature, relative humidity and necrophagous species accumulated to the experimental model used.

From the observation of all of the meat condition, since the dry fresh meat and dry frozen meat does not attract any necrophagous species except ants, also because of the insect colonization on the frozen meat was less compared to fresh meat, 2 more studies were conducted on the fresh meat at the same location. The fresh meat was used to provide reliable empirical data as fly is observed attracted more to fresh meat compared to other conditions of meat used.

Larva consumption study on the other hand, was conducted concurrently with the decomposition stages study since the equipment and the requirement do not change or disturb the existing experimental setup. The observation and all of the data from larva consumption evaluation were recorded for all of the meat conditions.

4.1.1 Location 1: Fresh Meat

Table 4.1 shows the decomposition stages, duration of decay and the entomological species for fresh meat.

Table 4.1 Decomposition stages, duration of decay and the entomological species for fresh meat

Days	Stages	Appearance	Entomological species
1	Fresh	Bright cherry - red colour meat, no smell, smooth and firm texture	Sarcophagidae (fly), Calliphoridae (fly), Phoridae (fly), Formicidae (black ant)
2	Fresh decay	Dark red colour, start to smell, dry soft texture	Sarcophagidae (fly), Calliphoridae (fly), Phoridae (fly), Formicidae (black ant)
3			Muscidae (fly), Formicidae (black ant)
4			Sarcophagidae (fly), Staphylinidae (beetle)
5	Advance decay	Dark brown red colour, strong smell, dry soft texture	Sarcophagidae (fly), Staphylinidae (beetle)
6			Sarcophagidae (fly), Staphylinidae (beetle)
7			Sarcophagidae (fly), Staphylinidae (beetle)
8			Sarcophagidae (fly)
9	Post decay	Dark brown, pungent smell, dry and hard texture	Sarcophagidae (fly)
10			Sarcophagidae (fly)
11			Sarcophagidae (fly)
12	Dry	Almost black colour	-
13 – 19 +			

The fresh meat on the 1st day placed at the location has a bright cherry - red colour meat, there is no smell with a smooth and firm texture. Even though there is no odour, the expected necrophagous insect which is flies is observed within several hours on the fresh meat and also black ants were seen attracted to the fresh meat. On the next day, the meat starts to smell and the colour turns to dark red colour with a dry soft texture. The odour increased on the 3rd day as the fresh decay stage continue.