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Influence of Maggot Mass Temperature In The Development of *Chrysomya megacephala* In Estimating Post Mortem Interval (PMI) in Kelantan, Malaysia.

Dissertation submitted in partial fulfilment for the Degree of Bachelor of Science in Forensic Science

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ACKNOWLEDGEMENT.

First and foremost, I would like to express the deepest appreciation to PM. Dr. P. T. Jayaprakash, my final year project supervisor, from the core of my heart for uplifting my spirits in the testing times and augmenting my motivations and besides for going all out of the way to provide me support and knowledge. Not to forget, for showing me a path of courage and success.

I would like to express my sincerest gratitude to Dr. Naji Arafat Mahat for his continually and convincingly conveyed spirit in supporting me during my practical and for giving me guidance in times of need.

From the abyss of my heart comes out the uninhibited gratefulness for my respected family for giving me inspiration, support and motivating me always.

Last but not least, thank you so much to all the persons involved in my final year project for the experience and opportunity given to me and most of all, thanks to all of you for being there with me always.

"Flatter me, and I may not believe you. Criticize me, and I may not like you. Ignore me, and I may not forgive you. Encourage me, and I may not forget you."

-William Arthur-

TABLE OF CONTENTS.

Tables **Page**

Figures

Appendix.

Appendix 11: Temperature recorded during the 3rd replicate in sunlit habitat on the sixth day-6.4.2011.

Appendix 12: Temperature recorded during the 3rd replicate in sunlit habitat on the sixth day-7.4.2011.

Appendix 13: Temperature recorded during the 4th replicate in sunlit habitat on the second day-12.4.2011.

Appendix 14: Temperature recorded during the 4th replicate in sunlit habitat on the third day-13.4.2011.

Appendix 15: Temperature recorded during the 4th replicate in sunlit habitat on the fourth day-14.4.2011.

Appendix 16: Temperature recorded during the 4th replicate in sunlit habitat on the fifth day-15.4.2011.

Appendix 17: Temperature recorded during the 4th replicate in sunlit habitat on the sixth day-16.4.2011.

Appendix 18: Temperature recorded during the 5th replicate in sunlit habitat on the second day-12.4.2011.

Appendix 19: Temperature recorded during the 5th replicate in sunlit habitat on the third day-13.4.2011.

Appendix 20: Temperature recorded during the 5th replicate in sunlit habitat on the fourth day-14.4.2011.

Appendix 21: Temperature recorded during the 5th replicate in sunlit habitat on the fifth day-15.4.2011.

Appendix 22: Temperature recorded during the 5th replicate in sunlit habitat on the sixth day-16.4.2011.

Appendix 23: Temperature recorded during the 1st replicate in shaded habitat on the second day-2.3.2011.

Appendix 24: Temperature recorded during the 1st replicate in shaded habitat on the third day-3.3.2011.

Appendix 25: Temperature recorded during the 1st replicate in shaded habitat on the fourth day-4.3.2011.

ABBREVIATIONS.

AT -Ambient Temperature

BT -Basket Temperature

GST -Ground Surface Temperature

BST -Body Surface Temperature

UIT -Underbody Interface Temperature

MMT -Maggot Mass Temperature

ABSTRACT.

The major contribution of forensic entomology is in the estimation of postmortem interval (PMI). Postmortem interval is the time elapsed since death, which can be used for narrowing down the suspect's presence at the time the body was disposed thereby linking the suspect with the crime. Determination of the PMI is based on two principles, one by studying the life cycle of a specific species of insect and the other by studying the succession of different species of insects.

As a poikilothermic (cold-blooded) organism, rate of growth of the larvae is a function of temperature and time. In this research, the influence of maggot mass temperature is evaluated as a factor affecting the rate of growth of the larvae. Maggot mass is referred to larvae that grow in groups. The larvae when in a mass produce heat that can be higher than the ambient temperature. In temperate countries, higher maggot mass temperature can sustain continual growth despite low ambient temperature. The range of variation between ambient temperature and maggot mass temperature in temperate countries has been reported to be 17°C to 33°C. Due to the higher maggot mass temperature, life cycle of insect is completed earlier. Research on the influence of maggot mass temperature on development of dipteran flies in tropical countries like Malaysia is lacking.

This research conducted at Universiti Sains Malaysia in Kelantan, Malaysia, a tropical environment evaluated the influence of maggot mass temperature in the development of *Chrysomya megacephala* in estimating the PMI in Kelantan, Malaysia. *C. megacephala* is chosen as it is the most prevalent fly of forensic importance in

Malaysia. The experiments were designed to cover both sunlit habitat and shaded habitat. The development of maggots in mass was studied in relation to the development of scarce maggots in the same environment.

The animal model decomposed was beef meat and as it was covered using a slotted plastic basket, the temperature inside the basket was considered the ambient temperature and five replicates of the experiment were carried out. The results indicated that the difference in the mean maggot mass temperature ranged from -0.1 to 6.9 °C when compared to the basket temperature.

The time taken for the larvae to attain the three instars which are the first, second and third was formal to be similar irrespective of whether they are in a mass or are sparse. However, the maggots in mass were always longer than there in sparse. The results indicate that maggot mean temperature has to be taken into consideration while estimating PMI.

KEYWORDS: Forensic entomology, *Chrysomya megacephala,* maggot mass temperature, postmortem interval (PMI) estimation, tropical examination in Kelantan, Malaysia.

INTRODUCTION.

Entomology is the scientific study of insects. Forensic entomology is an area that involves the study of arthropods which are associated with crime. It usually requires accurate identification of insects that are associated with human remains to determine time and place of death (Mullen and Durden, 2002). Zehner et. al., (2004) defined forensic entomology as the study of insects associated with corpse to determine elapsed time since death.

Maggots, flies and insects are valuable forensic indicators in establishing postmortem interval (PMI). PMI is defined as the period of time between death and corpse discovery (Catts and Haskell, 1990). Thus, knowledge on the species associated with decomposing corpses in different biogeoclimatic zones, their development rates with emphasis on development times of each immature stages (Turchetto et al., 2001) as well as lengths and weights of different larval stages (Wells and Lamotte, 2001) are essential in estimating PMI.

Additionally, the insects also provide information to the scientists and investigators on the relocation of body after death. This can be known when an insect collected from a corpse does not belong to the present location of the corpse but belongs to a different location. Such a finding would indicate possible movement of the body. Eventually, PMI also helps in identifying the suspect by eliminating those suspects whose locations were away from the place of recovery of the body and by connecting those suspects who were available at the estimated time of death. The

victim's identity can also be surmised by relating the PMI with the time the persons was reported missing (Catts, 1990).

There are several factors that can influence the development of flies and succession patterns of necrophagus species, such as geographical differences, effects of sun exposure, urban or rural scenarios, bodies inside buildings, effects of burial, bodies in water, bodies in vehicles, bodies in enclosed spaces, hung bodies, burned remains, and wrapped remains (Anderson, 2001).

Insects are poikilothermic organisms, also referred as to 'cold-blooded' organisms, that have no internal mechanism for regulating their body temperature. Thus, they may have either unregulated body temperatures or their behavior controls their temperature. Insects' have varying body temperatures and it is usually higher than the temperature of the environment, also known as the ambient temperature. Being a poikilotherm, a larva's rate of growth is essentially a function of temperature and time (Adams and Hall, 2003).

Although larvae are cold-blooded and generally must develop as a function of ambient temperature, the teeming, writhing mass of maggots that sometimes occurs during decomposition may produce significantly elevated temperatures (Greenberg, 1991). Peters (2003) has defined maggot mass as the larvae that grow in groups which can help enhance decomposition. These higher temperatures have an effect of shortening the developmental time of insects themselves in relation to ambient temperature. Thus, it is likely that maggot mass temperature may interfere with the estimation of PMI (Peters, 2003).

Maggot mass temperatures may exceed over 20°C above ambient temperatures (Deonier, 1940; Catts & Goffs, 1992). These studies were conducted in temperate countries where the ambient temperature was in the range of 17°C to 30°C. Under such temperatures, the difference between ambient temperature and maggot mass temperature is as high as up to 20°C. However, few studies have been conducted in tropical countries in determining the effect of maggot mass temperature in the development of blowflies. Ambient temperature in tropical countries being in the range of 25°C to 35°C, the differences between ambient temperature and maggot mass temperature required to be studied. Thus, whether or not maggot mass temperature influences development of blowflies in tropical countries is still unknown.

Deonier (1940) stated that the ambient temperature combined with the temperature of the masses of blow fly larvae furnished favorable conditions for larval development during cool weather. It has also been discovered that an exposed carcass decomposed earlier when compared with a shaded carcass (MacGregor, 1999). In a study, the temperature readings for the sun exposed site and the observed maggot masses were higher for most part throughout the day (Shean et al., 1993).

This research is the first of its kind conducted in Kelantan, Malaysia. It studies the influence of maggot mass temperature in the development of *Chrysomya megacephala* in estimating PMI. In this study, the developments of Chrysomya megacephala larvae growing in a mass and with larvae growing sparsely are compared. Beef meat left open in sunlit and shaded habitat was used to study the effect of maggot mass temperature on the development of *Chrysomya megacephala* between these two habitats.

REVIEW OF LITERATURE.

Post Mortem Interval (PMI).

PMI is defined as the time from death to discovery of the corpse; it is the most familiar use of entomological evidence in criminal investigations (Schoenly et al., 1996). Entomologists have applied developmental and successional data of carrion-associated arthropods to assist medicolegal investigators in cases of homicide, suicide and accidental death (Schoenly et al., 1996) because insects by their activities begin a biological clock that will allow for an estimation of the PMI (Goff, 1993). Determining the time of death is extremely important in a death investigation as it focuses the investigation into the correct time frame and eventually support or refute a suspect's alibi and improves the efficiency of the criminal investigation (Anderson, 1999).

Basically, the pathologists can estimate the time of death based on several medical parameters (Henssge et al., 1995) but these are only valid for the first few hours after death, becoming less valuable after that and usually not used beyond 72 hours. Therefore, forensic entomology is the most accurate and frequently the primary method in determining time of death when more than a day or two have elapsed (Kashyap and Pillai, 1989).

There are two ways of using insects in determining the PMI (Catts, 1990; Anderson, 1995). The first way is by heterotrophic succession of arthropod species as it occurs in predictable patterns during decomposition (Schoenly and Reid, 1987; Anderson and Van Laerhoven, 1996; Tantawi et al., 1996). Data from Hall and Doisy

(1993) indicated that dead bodies attract different assemblages of blow flies and flesh flies, depending upon the time elapsed since death.

The second way is by the knowledge on a given species to reach the stage of development that is collected from decomposing remains along with information on ambient temperature at the crime scene (Smith, 1986; Goff and Winn, 1997). The instar and length of the most mature maggots species can be determined and compared with data from laboratory rearing to see the duration for reaching that particular stage under controlled conditions (Goff, 2000). Eventually, it can be used in estimating PMI.

***Chrysomya megacephala* as a necrophagus fly.**

Many studies showed that the most important fauna for determining PMI are the blow flies (Nuorteva, 1977; Lord, 1990; Greenberg, 1991). Larvae of carrion flies, especially blowflies are by far the most common type of insect evidence collected during a death investigation (Catts and Goffs, 1992; Zehner et al., 2004). Most of the bodies are discovered in the first few weeks after death; blow flies are encountered more frequently and can reveal time of death more accurately than their successors (Greenberg, 1991).

In this study, *Chrysomya megacephala* is chosen because survey conducted by Lee et al., (2004) in Malaysia noted that among of the large numbers of fly species found on human cadavers, *Chrysomya* species were the most dominant of them all. *Chrysomya megacephala* has also been found predominantly in the work of Mahat et. al, (2009). Flies of *Chrysomya megacephala* species are large with size over 9.5mm long. The adults are bright metallic green with black margins on the second and third

abdominal segments, and have large red eyes almost touching each other. The face below the eyes is usually yellow to orange (Siriwattanarungsee et al., 2005).

Chrysomya megacephala has a life cycle of 4 growth stages, which are egg, larva, pupa and adult. The period from egg to adult usually takes 8 to 9 days. A female fly can lay from 150 to 300 eggs in each batch and the larva and pupa stages usually last about 4 days each (Sukontason et al., 2008). *Chrysomya megacephala* has a pronounced activity peak during the heat of the afternoon; this species is one of the first to become active early in the morning hours and is one of the last to depart carrion at nightfall. Due to their activity period, they are often the first species to arrive at dead humans as well as animal species, therefore are more often to deposit eggs (Byrd and Castner, 2010).

Maggot Mass Temperature.

A maggot mass is defined as the assemblage of feeding larvae in which metabolic heat increases the microclimate temperature above ambient (Byrd and Castner, 2010). The invading larvae become a feeding aggregation after partial penetration of the body (Greenberg and Kunich, 2002). Despite insects being poikilothermic or cold blooded and developing as a function of ambient temperature, the presence of maggot mass that sometimes occur during decomposition may produce significantly elevated temperature (Greenberg, 1991). The maggot mass temperature will be a function of the size of the maggot mass, stage of development, location of the corpse which has a relationship to ambient temperature (Catts and Goffs, 1992). A

mass of maggot with as few as 25 larvae was found showing temperature higher than the ambient temperature of 3 to 3.8°C (Greenberg and Kunich, 2002)..

However, in tropical countries the elevated maggot mass temperatures due to the presence of maggot mass have been rarely experimentally studied in order to completely understand the influence of maggot mass. Thermal energy of the larvae and carcass are very rarely discussed and researched. Many forensic entomologists (Greenberg 1991; Greenberg and Kunich 2002; Goff 2000; Byrd and Butler 1996; Byrd and Butler 1997; Campobasso et al. 2001; Hewadikaram and Goff 1991) mention the phenomenon of grouping of maggots and refer to the event as the massing of the maggots, coining the name maggot mass.

Greenberg (1991) demonstrated the thermal contribution of each larval instar in a maggot mass. First instar have little measurable effect, but second instar began to produce excess heat which peaked at 18°C above ambient, slightly before third instars reached maximum size. The temperature falls rapidly when post feeding larvae disaggregate. Therefore, in a heavy infestation, the developmental rate of second instars and actively feeding third instars should be calculated at a higher temperature than ambient. This could reduce the PMI estimation by more than a day depending on the ambient temperature (Greenberg, 1991).

Limited research had been done on the thermal energy of the maggot mass and maggot mass temperature has not been incorporated into determination of post mortem interval yet (Peters, 2003). The probable impact of the maggot mass heat generation phenomenon on development rates, and ultimately on determination of the PMI,

demands further studies (Catts, 1992). Thus, in this study, the larval development between those larvae growing in a mass and those growing sparsely was compared.

Effect of geographical regions.

Temperature, seasons and habitats define a geographical region and may influence the insect community within that area (Dillon and Anderson, 1997). This geographical diversity contributes to difficulties in estimating PMI due to physical factors in the environment, such as temperature, wind and rainfall or humidity. These may greatly alter the gross appearance of decomposing remains with similar post mortem interval but in different habitats (Goff et al., 1988).

Catts (1992), during his studies using pig as a model in fall in Washington, U.S.A. found that maggot mass temperatures exceeded subfreezing ambient temperatures by as much as 35 (°C); even tough carcass temperatures were only 8°C above the low ambient temperature. He also mentioned that regardless of summer or fall, maggot mass temperatures ranged as much as 35°C to 45°C higher than ambient low temperature and about 20°C above ambient high temperature. However, this is reported in temperate country where there are four seasons and the ambient temperature range is larger between 17°C to 30°C.

No research has been conducted in tropical countries like Malaysia. Kota Bahru, Kelantan has ambient temperature range of 23°C to 31° (Malaysian Meteorological Department, 2011) and the lowest temperature here is higher than in temperate countries. Therefore, the difference between the ambient temperature and maggot mass temperature in tropical countries deserves experimental study.

Effects of sun exposure vs. shade.

Effect of sun exposure must be considered as it can increase the temperatures that can effect developmental times (Greenberg & Kunich, 2002). Blow flies required sunlight and warmth to oviposit (Greenberg, 1990). Bodies found in direct sunlight are warmer, heating up more rapidly and decomposing faster. They lost biomass more rapidly than bodies found in shade and progress through decompositional stages faster (Reed, 1958; Shean et al., 1993; Dillon, 1997; Dillon and Anderson, 1995, 1996).

In a comparison of pig carcasses placed in sun and shade in West Virginia, carcasses in sunlit habitats decomposed faster than those in shaded habitat. Maggot mass temperatures positively correlated with ambient temperatures in sunlit habitats but not in shaded habitats (Joy et al., 2006). In Malaysia, Mahat et. al., (2009) reported the differences in the development time in *Chrysomya megacephala* between sunlit and shaded habitats.

In warmer temperatures and high level of moisture, insects are known to grow faster. The opposite conditions have also been reported to retard insect growth significantly (Anderson et al., 2000). In general, within a certain range of temperatures, development increased as the temperature increased and may become lethal to the insect in extreme temperature. This factor needs further research to elucidate its real influence on larval development.

The Choice of Host.

Appropriate selection of animal model is important in forensic entomology research. Several different types of animal models have been in conduct entomological research, with pig being the most common (Cianci and Sheldon, 1990; Hewadikaram and Goff, 1991; Goff, 1992; Shean et al., 1993; Komar and Beattie, 1998; Goff, 2000; Carvalho et al., 2000; Anderson, 2001; Watson and Carlton, 2003). Others include domestic cats (Early and Goff, 1986), human cadavers (Rodriquez and Bass, 1983; Goff et al., 1988; Mann et al., 1990; Anderson, 1997; Grassberger and Reiter, 2001; Barreto et al., 2002), rabbit carcasses (Denno and Cothran, 1976; Goff et al., 1989; Goff et al., 1991; Wells and Greenberg,, 1992; Goff et al. 1997; Mahat, 2009), leghorn chicken hens (Hall and Doisy, 1993), laboratory rats (Greenberg, 1990); beef meat (Pritam and Jayaprakash, 2009) alligator, deer and bear (Watson and Carlton, 2003).

Carcass types and size can have different effect on decomposition rate (Denno and Cothran, 1975; Hewadikaram and Goff, 1991), species composition (Schoenly and Reid, 1983) and insect succession (Wells and Greenberg, 1994). Additionally, carrion flies that coexist within a regional and a local scale have different oviposition preferences to certain kinds of carcasses as means of resource partitioning (Davies, 1990; Wells and Greenberg, 1994). Thus, care must be taken when applying data from one carcass type to estimating the PMI of another type.

For this study, fresh beef meat weighing 500 gram was used as the choice of host or animal model since this research is restricted only to development of *Chrysomya megacephala* and concerns obtaining maggot mass in controlled condition.

Collection, Killing and Preservation of Larvae.

The spiracle indicates the stage of maggots. However, when maggots are not preserved correctly, the exact length cannot be recorded and the posterior spiracles can be difficult to observe and identify. Most plots of larval growth described the change in body length with age. Typically, larvae are killed and preserved in some fluid prior to measurement. Tantawi and Greenberg (1993) found that the kind of solution in which maggots are killed or preserved has a significant effect on their length and therefore their estimated age and thus can lead to a miscalculation of the PMI.

A uniform and standardized technique of preservation must be applied to preserve maggots and to prevent shrinkage and deformation. Tantawi and Greenberg (1993) prescribed that the most appropriate way to preserve maggot specimens was by fixing their internal protein by placing them in boiling water for approximately 10 seconds.

However, Adams and Hall (2003) have suggested that the larva should be killed by immersion in actively boiling water for not less than 30 seconds, measured immediately and then transferred to 80% ethanol for preservation. The positive aspects of this methods are that the methodology is simple; measuring the larvae immediately after killing avoids any post mortem changes in length that might occur once the larvae are placed in preservative, 80% ethanol has low toxicity and readily available, and using actively boiling water would ensure that all larvae are immersed in water at the same temperature and processing the larvae in this way always resulted in good preservation.

OBJECTIVE OF THE STUDY.

The general objective in this research is:

i. To determine the effect of maggot mass temperature in the development of *Chrysomya megacephala* and eventually in estimation of PMI.

The specific objectives in this research are:

i. To determine the effect of maggot mass temperature in the development of *Chrysomya megacephala* in sunlit habitat and shaded habitat.
ii. To determine the development of *Chrysomya megacephala* when the maggots are sparse in sunlit and shaded habitat.
iii. To determine the differences in the maggot mass temperature in relation to the different larval stages of *Chrysomya megacephala*
iv. To compare the maggot mass temperature reported in temperate countries with the one observed in Malaysia (a tropical country).
v. To infer the influence of maggot mass temperature in estimating of PMI in Kelantan, Malaysia.

MATERIALS AND METHODS.

I. Materials used in this research.

Host selection.

Host selection acquires importance because the validity of the result or baseline data obtained is applicable to real case situation. It is also important host selected has to simulate a fresh body and follow decompositional stages as closely as possible to the real situation. Therefore, fresh beef meat weighing 500 gram was used as this study as it is limited only to study the influence of maggot mass temperature in development of *Chrysomya megacephala*. In addition, beef meat weighing 500 gram is enough for completing the development of *Chrysomya megacephala*.

List of Chemicals and Reagents Used.

1. 80% ethanol.
2. Ethyl acetate.

List of Equipments Used.

1. Latex gloves.
2. Pointed forceps.
3. Beakers.
4. Spatula.
5. Digital probe thermometer (UEI PDT550 Digital Thermometer).
6. Petri dishes.
7. Surgical blades.
8. Permanent marker pens.
9. Notebook and stationery.
10. Plastic container tubes 40 ml.
11. Cotton balls or gauges.
12. Small pins of 3cm length.
13. Double cellotape.
14. Fly proof nets.
15. Plastic baskets (16 inch x 20 inch) with slotted openings.
16. Plastic baskets (13 inch x 18 inch).
17. Rubber bands.
18. Tissue napkins.
19. Sticker labels.

During daily observations, a digital camera, Fujifilm Finepix J10, was used for photographing the specimens.

II. Research methodology.

Choosing the site of experiment.

One of the objectives of this study is to compare the effect of maggot mass temperature on the development of *Chrysomya megacephala* larvae between sunlit habitat and shaded habitat. Therefore, for sunlit area, open field in front of Animal

Research and Service Center, USM was chosen as it provides about 12 hours of sunlight without interference of shadows from trees or buildings. For shaded area, parking lot of administration building of Health School USM was chosen as the experiment site as it has no ray of sun entering the parking lot, yet is open on sides.

Description of the cycles of experiments and its replicates.

The experiment was planned in five replicates for each of the sunlit and shaded habitats. Each replicate involved decomposition of two lumps of beef meat, one in sunlit habitat and the other in shaded habitat. Thus, a total of 20 animal models were decomposed. Although, the sunlit habitat and shaded habitat replicates were planned to be conducted simultaneously, due to heavy rains during some of the period of the replicates, the sunlit habitat and shaded habitat experiment could not be carried out simultaneously. During such period of heavy rains, the replicates of shaded experiment were continued and the replicates for sunlit experiment had to be postponed.

The duration of the replicate are provided in the below table:

Replica	Sunlit habitat	Shaded Habitat
1	1.3.2011 to 6.3.2011	1.3.2011 to 7.3.2011
2	1.4.2011 to 7.4.2011	8.3.2011 to 15.3.2011
3	1.4.2011 to 7.4.2011	19.3.2011 to 26.3.2011
4	11.4.2011 to 16.4.2011	26.3.2011 to 2.4.2011
5	11.4.2011 to 16.4.2011	26.3.2011 to 2.4.2011

Preparing the beef meat.

Fresh beef meat was bought early in the morning, at 10 a.m. on the same day the experiment was conducted and was placed at the site of experiment within 30 minutes after purchase. The meat was purchased from Mydin Supermarket, opposite USM, a closed one where flies do not swarm as in the open markets and was transported in plastic bags sealed with sticky tape. The meat was left open on the ground and was covered with slotted basket for enabling the flies to reach the meat and lay eggs, and to prevent other animals from scavenging the meat. For each site of experiments, two 500 gm lumps of fresh beef meat were left in that area with an inter distance of more than 10 meters for ovipositioning by *Chrysomya megacephala.*

Oviposition of the *Chrysomya megacephala.*

Once the beef meat was left for decomposition, the site was visited once in every four hours until eggs were observed. After the observation of oviposition, the sites were visited every two hours. The beef meat revealing bigger cluster of eggs was treated as the one for maggot mass and the one that revealed eggs that were sparse was meant for rearing maggot in sparse. When the eggs were in masses in both of the meat, the mass of lesser size was transferred to the meat with bigger mass. Among all the sites of experiment involving a pair of beef meat, mass of egg was always found in at least one beef.

Preparing the Maggot Mass.

After the appearance of larvae, it must be ensured whether they were in a group or not. In one or two occasions, the larvae in beef meat labeled as maggot mass were not enough to form mass as the eggs might have been lost due to predation by ants. Then the required number of larvae from the other beef meat (scarce larvae) was transferred to the beef meat labeled as 'maggot mass'.

During the experiment in sunlit habitat in first replicate, the meat was left on grassy surface and once the larvae migrated, the pupae could not be traced. This may be due to the thick grassy surface. To prevent the loss of pupae, another normal plastic basket of lesser size (13 inch x 18 inch) was used for holding loose soil about 1 inch deep. The meat was placed on top of the soil and the plastic basket was covered with slotted plastic basket. In addition, the slotted plastic basket was covered with fly-proof nets. Once this was done, the migrating larvae pupariated in the soil and could be observed without fail. The purpose of this step was also to prevent further oviposition by *Chrysomya megacephala* and other species as this would interrupt the development of larvae with more of younger generation producing food competition. Next, the plastic baskets with beef meat of covered with slotted basket experiment set up covered with plastic sheet as to prevent rain water from pouring inside the baskets but yet also to allowed sunlight coming through. This arrangement was necessitated since it was intermittently raining.

Temperature Reading.

Once the freshly hatched larvae was observed, temperature reading was taken at every two hours between 7 a.m. to 7 p.m. After the fourth day, the temperature reading was taken between every four hours. The interval of two hours prior to the first four days was considered reasonable since this study relates to maggot mass and the temperatures. The several temperature readings documented were:

1. Ambient air temperature recorded by readings taken about 1 feet from side of the basket.
2. Ambient air temperature inside the basket taken by placing the thermometer inside the basket.
3. Ground surface temperature obtained by placing the thermometer on the ground on top of the soil surface inside the basket.
4. Body surface temperature obtained by placing a thermometer on the upper surface of the body. The thermometer's probe was in contact with the upper surface of the meat.
5. Underbody interface temperature obtained by sliding the thermometer between the body and the ground surface.
6. Maggot mass temperature obtained by gently inserting the thermometer into the center of the maggot mass. It must be noted that the thermometer was maintained motionless after insertion into the mass and while reading the temperature since any movement of the probe would disrupt the larval mass.

Caution was taken not to allow direct rays of the sun to shine on the sensing element of the thermometer. The radiant heat from the sun would cause excessive readings when compared with the true ambient temperatures. The thermometer was always shaded from direct sunlight while obtaining temperature data.

Observation, Collection and Preservation of Larvae.

Three to five larvae were sampled from both the beef meat with maggot mass as well as sparse maggots everyday. The larvae collected with forceps were placed into petri dish for each site of experiment. Then, the larvae were killed by immersing in boiling water for about 30 seconds and their morphology was observed. The larvae were then transferred into 80% of ethanol for preservation (Adams and Hall, 2003) and kept in labeled plastic container tubes 40ml.

Few of the pupae from each site of locations were collected for rearing and placed in a 100ml beaker half filled with soils and covered with fly proof nets. After recording the appearance of adult flies in the beaker, the adults were killed using a few drops of ethyl acetate soaked in a cotton ball or gauze (Dahms et al., 1979). The adult flies were later pinned onto a styrofoam board for identification and preservation.

Identification of *Chrysomya megacephala.*

Species of maggots were identified by observing the morphology of posterior spiracle at the caudal segment. The identification of instar was also done by observing the morphological variations of the posterior spiracle which represent the stage of growth (Byrd and Castner, 2001). Spiracles are useful to determine the larval instar

which were categorized into three, "first larva" was between hatching and the first moult; "second larva" between first and second larval moults and "third larva" between the last larval moult and pupation (Centeno et al., 2002). The caudal segment was observed under a microscope and the identification was based on the standard description in literature (Sukontason et al., 2008).

The posterior spiracles is in pair which has only one spiracular opening that presents a pair of ventromedial tubercles and a pair of anal tubercles, incomplete peritreme and slightly pigmented. The posterior pair of spiracles is situated in a convex position in the median-apical region of the spiracular plate.

The posterior spiracle of first instar larvae can be observed at the posterior end of segment 12. On the second instar, the formation of posterior spiracle can be observed on the second instar cuticle with two still incipient spiracular openings. In this segment, two pairs of tubercles can be seen in the dorsal part. For the third instar, pair of posterior spiracles with two openings each, peritrema closed and pigmented and located in a convex position in the medial-apical region of the spiracular plate.

RESULTS.

The entire raw data for the first replica in sunlit habitat recorded during every visit are provided in Tables 1 to 5 while, the mean values are presented in Tables 6 and Figure 1. The raw data for replicate 2 to 5 in sunlit habitat and replicate 1 to 5 in shaded habitat are provided in the appendices. The daily mean temperature for all the experiments conducted in sunlit habitat in replicates 2 to 5 are presented in Tables 7 to 10. The corresponding graphical presentations of the data are shown in Figures 2 to 5. The daily mean temperatures for all the experiments conducted in the shaded habitat in replicates 1 to 5 are presented in Tables 11 to 15. The corresponding graphical presentations of the data are shown in Figures 6 to 10.

The duration in hours taken for each development stage in all the five replicates in sunlit habitat are abstracted in Table 16 and its graphic representation is provided in Figure 11. The duration in hours taken for each development stage in all the five replicates in shaded habitat are abstracted in Table 17 and its graphic representation is provided in Figure 12.

Date	Time	Situation	AT (°C)	BT (°C)	GST (°C)	BS (°C)	UIT (°C)	MMT (°C)
2.3.2011	11:00 AM	Open and sparse	35.3	34.5	32.0	31.1	30.3	x
2.3.2011	11:15 AM	Open and maggot mass	35.3	35.2	31.7	32.5	31.3	32.1
2.3.2011	1:00 PM	Open and sparse	33.9	36.2	35.2	33.7	32.7	x
2.3.2011	1:15 PM	Open and maggot mass	34.2	37.7	37.4	35.3	33.4	34.7
2.3.2011	3:00 PM	Open and sparse	33.7	33.6	35.2	33.5	33.9	x
2.3.2011	3:15 PM	Open and maggot mass	35.2	35.7	35.6	32.6	33.8	35.1
2.3.2011	5:00 PM	Open and sparse	31.2	31.4	33.1	32.6	32.3	x
2.3.2011	5:15 PM	Open and maggot mass	31.6	31.4	31.5	33.0	34.3	34.2
2.3.2011	7:00 PM	Open and sparse	28.6	27.4	29.9	27.5	31.4	x
2.3.2011	7:15 PM	Open and maggot mass	28.0	27.8	29.1	28.8	31.8	31.3

Table 1: Temperature recorded during the 1st replica in sunlit habitat on the second day-2.3.2011.

Date	Time	Situation	AT (°C)	BT (°C)	GST (°C)	BS (°C)	UIT (°C)	MMT (°C)
3.3.2011	7:00 AM	Open and sparse	24.9	23.1	25.6	26.8	31.1	x
3.3.2011	7:15 AM	Open and maggot mass	24.2	24.1	26.0	25.6	28.9	31.1
3.3.2011	9:00 AM	Open and sparse	28.0	27.4	27.1	32.1	34.8	x
3.32011	9:15 AM	Open and maggot mass	29.3	29.4	27.5	29.4	30.1	33.3
3.3.2011	11:00 AM	Open and sparse	33.2	35.4	32.7	36.2	38.9	x
3.3.2011	11:15 AM	Open and maggot mass	35.1	35.8	30.7	36.5	35.1	39.2
3.3.2011	1:00 PM	Open and sparse	35.2	39.8	44.1	39.3	41.7	x
3.3.2011	1:15 PM	Open and maggot mass	35.8	41.0	41.7	37.4	37.5	39.9
3.3.2011	3:00 PM	Open and sparse	34.3	42.4	40.9	41.0	41.5	x
3.3.2011	3:15 PM	Open and maggot mass	34.9	43.1	44.3	39.5	40.2	41.1
3.3.2011	5:00 PM	Open and sparse	31.0	36.0	36.9	37.2	39.8	x
3.3.2011	5:15 PM	Open and maggot mass	31.3	35.0	35.4	34.5	39.8	40.0
3.3.2011	7:00 PM	Open and sparse	28.1	28.0	29.3	31.5	36.2	x
3.3.2011	7:15 PM	Open and maggot mass	28.0	27.9	28.9	30.6	34.3	36.0

Table 2: Temperature recorded during the 1st replica in sunlit habitat on the third day-3.3.2011.