CHAPTER 1

INTRODUCTION

1.1 FORENSIC ENTOMOLOGY

The area of forensic entomology has developed in recent years to become an increasingly important aspect of the forensic sciences. Forensic entomology is the broad field where arthropod science and the judicial system interact. It has been subdivided into three principal areas focused on those issues most often litigated, which are urban entomology, stored products entomology and medico-legal entomology (Lord & Stevenson, 1986).

The use of insects and other arthropods as evidence in criminal investigations dates from 13th century in China (McKnight, 1981). The first modern forensic entomology case report to include an estimation of postmortem interval (PMI) was given by the French doctor Bergeret in 1855 (Bergeret, 1855). Though the result of his investigations was incorrect, it demonstrated that insect life cycle could be applied to help determine the post mortem intervals of the deceased, and paved the way for further work in forensic entomology (Hall, 2001). It was Megnin (1894) that focused western attention on the potential of arthropod science in forensics by his pioneering work studying the species, stage of growth and life cycle of fly recovered from the cadavers. Gaining much attention, this subject was extensively studied in the west (Leclercq, 1969; Easton & Smith, 1970; Keh, 1985; Smith, 1986; Catts & Goff, 1992; Hall, 2001; Greenberg & Kunich, 2002).

The decomposition of cadavers is divided into several stages, namely, fresh stage, bloated stage, decay stage, advance decay stage and dry / remains / skeletal stage.

Although there have been many decomposition studies conducted in different parts of the world and under different environmental conditions, most studies have been conducted in temperate areas and few in tropical and subtropical habitats (Goff, 2003).

In Malaysia, Reid (1953) was the first to mention the use of forensic entomology to determine the PMI in a female corpse found in Penang in 1953. Lee (1989) examined specimens sent by police departments and reviewed the occurrence of fly in forensic cases. There is a knowledge gap especially on the biology and bionomics of the insects recovered from the cadavers and hence no complete study has been conducted on the wave of succession of arthropod fauna on carrion under different ecological habitat and conditions in Malaysia.

In the present research, aspect of medical–legal entomology was studied. The medico-legal section focuses on the criminal component of the legal system and deals with the necrophagous feeding insects that typically infest on human remains.

1.2 THE IMPORTANCE OF PRESENT STUDY

Little information was available on flies and maggots collected from carcasses in indoor and different ecological habitats. So far no study has been conducted on insect succession on carcasses in indoor conditions in Malaysia. This is the first study on insect succession of carcasses in indoor conditions in Malaysia. In addition, most of the forensic entomological studies in Malaysia were conducted in oil palm plantation (Heo *et al*., 2007, 2008a, 2008b, 2009; Azwandi & Abu Hassan, 2009), rubber tree plantation (Omar *et al*., 1994a), secondary forest (Omar *et al.*, 1994b) and urban (Ikhwan, 2004) areas with mean temperatures ranging from 25ºC to 30ºC. Thus, this study attempted to investigate the fauna succession and decomposition rate of carcasses in three different ecological habitats, namely lowland forested area (mean temperature between 25ºC to

27ºC), coastal area (mean temperature between 30ºC to 33ºC) and montane forested area (mean temperature between 20ºC to 21ºC). This baseline information pertaining to knowledge of development of forensically important flies is essential to determine the Post Mortem Intervals (PMI) of medico-legal cases in different ecological sites in Malaysia.

This study hopefully results in fly and maggot databases and protocols for forensic entomology in the Malaysian context to be used by departments involved in forensic entomology.

This study promotes the recognition of forensic entomology as an important component of the criminal justice system, and encourages a high level of scientific and professional rigour in the field of forensic entomology, by maintaining and encouraging standard methods and practices in Malaysia. The potential beneficiaries of these research findings will be Ministry of Health, pathology department, police department, universities and the general public.

1.3 AIMS OF STUDY

The aims of the study were:

(i) To determine the arthropod succession in different ecological habitats, namely lowland forested area (Ulu Gombak, Selangor), coastal area (Tanjong Sepat, Selangor) and montane forested area (Genting Highland, Pahang),

(ii) To determine the arthropod succession on carcasses placed in indoor and outdoor conditions, and

(iii) To determine the arthropod succession on carcasses being incinerated, submerged in freshwater river and treated with insecticide and its effect on PMI estimation.

1.4 SCHEME OF STUDY

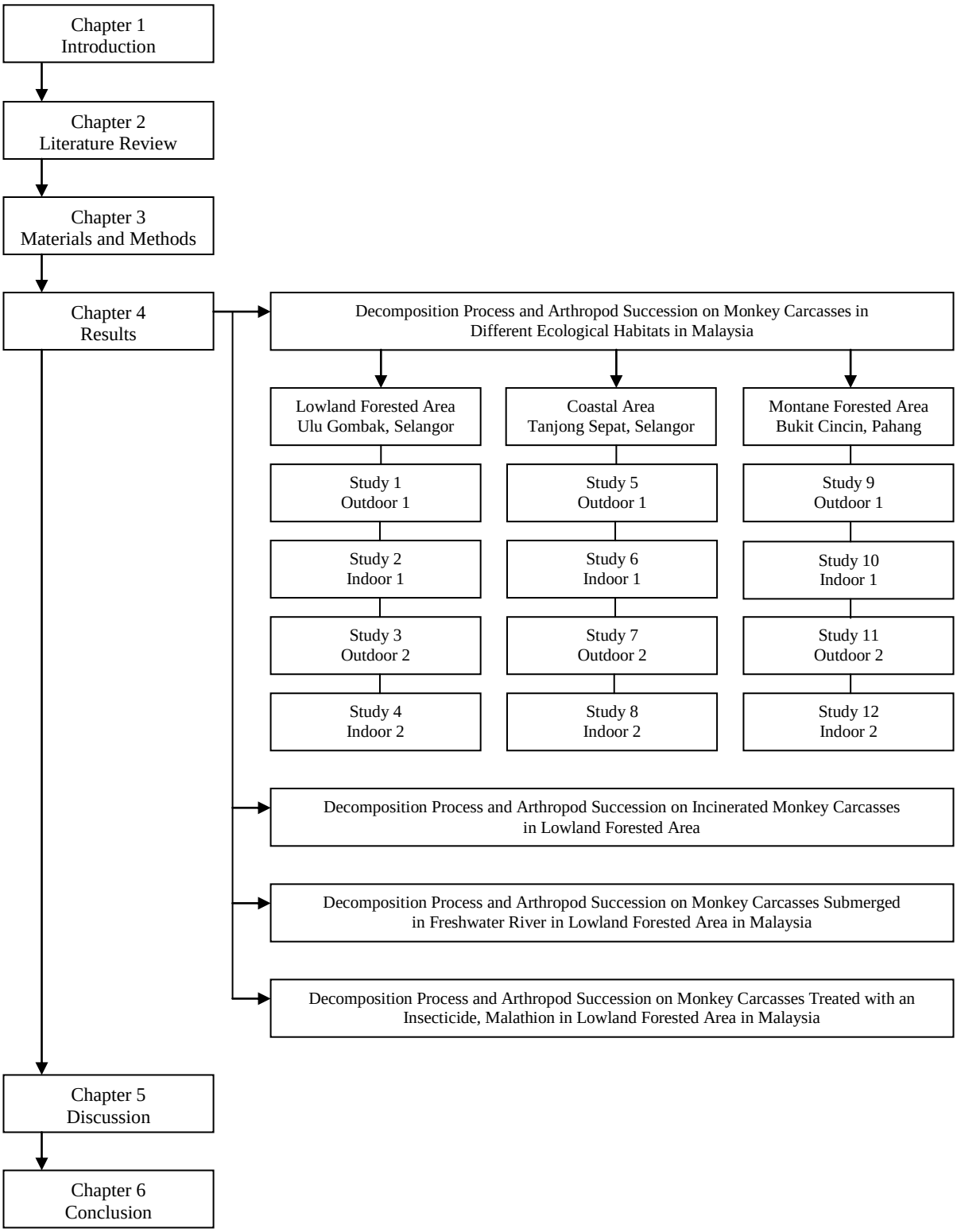

Figure 1.1. Schematic diagram of "Studies on forensically important entomological specimens recovered from monkey carrions exposed to different ecological habitats in Malaysia".

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CHAPTER 2

LITERATURE REVIEW

2.1 THE BEGINNING OF FORENSIC ENTOMOLOGY IN THE WORLD

The first documented forensic entomology case was reported in China in the 13th century. In 1247, Sung Tz'u, a Chinese lawyer and death investigator, published *The Washing Away of Wrongs*, a training manual for death scene investigators. In this medico-legal book, he related a murder investigation in a rural, agricultural village. A man was found dead on the road with numerous slash wounds in his head that appeared to have been made by a sickle. Sung Tz'u assembled the men of the village and lined them up with their sickles on the ground in front of them. It was summer and flies were numerous. Sung Tz'u confronted the sickle's owner with the fact that his was the only sickle with flies clustered on it, and the man confessed (Benecke, 2001; Greenberg & Kunich, 2002).

It was not until the mid-1800s that medico criminal entomology saw recorded use in the West. In 1831, the famous French medical doctor Orfila observed a large number of exhumations; he understood that maggots play an important role in the decomposition of corpses (Benecke, 2001). The first modern forensic entomology case report to include an estimation of postmortem interval (PMI) was given by the French doctor Bergeret in 1855 (Benecke, 2001).

In 1894, Megnin published *La Fauna des Cadavres*. In it, he expended his former theory of four waves for freely exposed corpses to eight successional waves. The book dealt with larval and adult forms of a number of families, and its drawings focused on wing venation, posterior spiracles and overall anatomy of the insects for

identification. Megnin also described 19 case reports, including his own cases between 1879 and 1888 (Megnin, 1894).

After the French publication of Megnin's popular book on the applied aspects of forensic entomology, the concept quickly spread to Canada and the USA. At the time, researches recognized that the lack of systematic observations of forensically important insects stood in the way of their use as indicators of postmortem interval. General advances in insect taxonomy and ecology helped close this gap over the following decades.

From 1960s to the 1980s, Leclercq (1968) and Nuorteva *et al.* (1967; 1974) were primarily responsible for maintaining the method in Central Europe, with focus on case work. Since then, basic research in the USA, Russia and Canada has opened the way to the routine use of entomology in forensic investigations (Benecke, 2001).

Nevertheless, these works were mostly conducted in Europe, the USA and Canada, where conditions are vastly different from the tropics such as countries like Malaysia.

2.2 THE INITIAL AND REVIEW ON FORENSIC ENTOMOLOGICAL CASES ON HUMAN CORPSES IN MALAYSIA, 1950s – 2000s

In Malaysia, Reid (1953) from Institute for Medical Research (IMR), Kuala Lumpur published a book titled *Notes on houseflies and blowflies in Malaya*. In it, he documented the first forensic entomology case of Nevin in Penang, who found *Chrysomya megacephala* (Diptera: Calliphoridae) larvae in a dead woman in 1950. His book also published notes on flies of forensic importance.

However, there was no report on forensic entomology cases in the following 30 years till Lee & Cheong (1982) reported the recovery of a larva of *Hermetia* sp. (Diptera: Stratiomyidae) from a highly decomposed female body in 1982.

Since 1950, Institute for Medical Research (IMR) has accumulated the largest collection of forensic entomology cases. Despite a wealth of information, however, there has been no known comprehensive review of these collections. In 1984, the first comprehensive review on all forensic entomological cases (1973 – 1983) received by Medical Entomology Unit, IMR was done by Lee *et al*. (1984). Besides, Lee *et al*. (1984) also reported some considerations on specimens handling and preservation techniques. Since then, Lee (1989, 1996a, 1996b) reviewed and updated some of the IMR collection of fly maggots recovered from human cadavers from time to time.

Lee (1994) documented the larvae of the drone fly, *Eristalis* sp. (Diptera: Syrphidae) recovered for the first time from a decomposed corpse of a new-born baby found floating in an irrigation canal. Thus, his report confirmed the association of an aquatic environment with *Eristalis* sp. larvae in Malaysia.

On the other hand, Omar *et al*. (1994a) reported the first record of *Synthesiomyia nudiseta* (Diptera: Muscidae) in Peninsular Malaysia and its apparent involvement in decomposing corpses found indoors. Their finding was useful in forensic investigations for the determination of death intervals of bodies found inside buildings.

Hamid *et al*. (2003) and Salleh *et al*. (2007) reviewed forensic entomology cases from Kuala Lumpur Hospital and Hospital of Universiti Kebangsaan Malaysia in year 2001 and 2002, respectively, and reported that *Ch. megacephala* was the dominant decomposer on human cadavers in Malaysia.

In 2004, another comprehensive review of the forensic entomological specimens received by Unit of Medical Entomology, IMR, from Hospitals and the police in Malaysia in the last 3 decades (1972 – 2002) was done by Lee *et al*. (2004). According

to Lee *et al*. (2004), a total of 448 forensic entomological cases were received from 1972 – 2002. From these, 18 species of cyclorrphaga flies were identified (Table 2.1). *Chrysomya megacephala* and *Chrysomya rufifacies* were the commonest species found in cadavers from different ecological habitats. Lee *et al*. (2004) reported that a total of 73.44% cases had single fly infestation, 24.33% cases had double fly infestation and 2.23% had triple fly infestation. They concluded that although a large number of fly species were found on human cadavers, the predominant species are still those of the genus *Chrysomya*.

In 2008, Kavitha *et al*. conducted a study to compare the estimated post-mortem intervals cases by pathologist and entomologist in Malaysia. Their finding shows that the methods used by the entomologist and pathologist for estimating post-mortem interval were significantly correlated, at 0.01 level. Scatter plot analysis confirmed that maggot body length increases over time until 4 – 5 days, after which it starts to decrease over time (6 – 8 days).

Nazni *et al*. (2008) reported the first recovery of *Piophila casei* (Diptera: Piophilidae) from 2 human cadavers found indoors in Malaysia. Nazni *et al*. (2008) concluded that *Piophila casei* is an important forensic indicator because larvae of this species were found during active decay and skeletonized stages.

Kumara *et al*. (2009a) reported that the adults and larvae of the beetle *Dermester ater* (De Geer) were found to be infesting a human corpse at an advanced stage of decomposition. The corpse was found in a house in the residential area of Bukit Mertajam, Penang, and the estimated time of death was 14 days prior to the discovery of the body based on the police investigation. Kumara *et al*. (2009a) highlighted that the infestation of dermestid beetles can occur within 2 weeks on the corpse in the tropical climate of Malaysia. However, for an accurate estimation of post-mortem interval using

Dermester ater larvae in Malaysia, further research into life cycle of *Dermester ater* in tropical environment should be conducted (Kumara *et al*., 2009a).

Table 2.1. Fly and non-fly specimens recovered from 448 forensic entomological cases from 1972 – 2002 in Malaysia (Lee *et al*., 2004).

Specimens	Percentage (%)
Order: Diptera	
Calliphoridae	
Chrysomya megacephala Fabricius	47.99
Chrysomya rufifacies Macquart	29.46
Chrysomya villeneuve Patton	2.23
Chrysomya nigripes Aubertin	1.56
Chrysomya bezziana Villeneuve	0.89
Chrysomya pinguis Walker	0.22
Chrysomya sp. Robineau-Desvoidy	10.49
Calliphora sp. Robineau-Desvoidy	5.36
Hemipyrellia ligurrien Wiedemann	0.67
Hemipyrellia sp. Townsend	0.45
Lucilia sp. Robineau-Desvoidy	4.69
Sarcophagidae	
Sarcophaga sp. Meigen	6.25
Muscidae	
Ophyra spinigera Stein	0.22
Ophyra sp. Robineau-Desvoidy	1.34
	0.22
Synthesiomyia nudiseta Van Der Wulp	
Stratiomyidae	
	0.22
Hermetia illucens Linnaeus	
Hermetia sp. Latreille	3.35
Syrphidae	
Eristalis sp. Latreille	0.22
Order: Phthiraptera	
Pthiridae	
Pthirus pubis Linnaeus	0.45
Order: Coleoptera	
Unidentified Beetles	0.22

2.3 FORENSIC ENTOMOLOGICAL AND FAUNA SUCCESSION STUDIES ON NON-HUMAN PRIMATES IN MALAYSIA

The first study on fauna succession on monkey (*Presbytes cristata*) carcasses had been conducted in Malaysia by Lee & Marzuki (1993). Their study focused on the occurrence of carrion-related arthropods especially the flies related with monkey carcasses either fully exposed or partially buried near the fringes of tropical forest in Ulu Gombak, Selangor. In this study, the decomposition of carcasses was divided into 4 stages (fresh, decay, dry and remains) based on physical changes. However, no bloating stage was observed in this study, because to the internal organs of the monkeys were removed for other studies and parts of the intestines were exposed due to the dissection wounds.

The second study on fauna succession on a monkey (*Macaca fascicularis*) carcass was conducted by Omar *et al.* (1994b) in a rubber tree plantation at Sungai Pusu, Gombak, Selangor. They reported that the carrion had gone through 5 stages of decomposition, namely fresh, bloated, decay, post-decay and dry remains, and the calliphorids were the earliest to colonise, followed by the muscids and stratiomyiids. Omar *et al.* (1994b) also reported that the presence of *Hermetia illucens* (Diptera: Stratiomyidae) and *Ophyra spinigera* (Diptera: Muscidae) larvae or pupae on dead human bodies could be indicative of long post-mortem interval.

Due to very little information on the behaviour or bionomics of Malaysian sarcophagous flies, Omar *et al*. (1994c) conducted a study to elucidate the patterns of arthropod succession in monkey and cat carcasses at 5 different sites in Selangor and Kuala Lumpur. Omar *et al*. (1994c) reported that *Hemipyrellia ligurriens*, *Hypopygiopsis* sp. and *Lucilia sinensis* preferred to lay their eggs on the nose orifices and mouth of the carcasses, while *Chrysomya* sp. preferred to oviposit their eggs all over the body, amongst the furs of the animals. Omar *et al*. (1994c) also reported the

dispersal behaviour of post-feeding larvae of Malaysian native flies, and summarised as (1) late third-instar larvae of *Chrysomya rufifacies*, *Chrysomya villeneuve*, *Chrysomya chain*, *Chrysomya nigripes* and *Ophyra spinigera* remain on or near the carcasses, and (2) *Chrysomya megacephala*, *Chrysomya pinguis*, *Lucilia sinensis* and *Hemipyrellia ligurriens* are the most dispersive and pupate in the soil far from the carcasses. This study provided useful information to the forensic entomologist in the crime scenes, which may possibly miss the first wave of maggots if sampling had been confined only to the larvae and puparia found on the body and some of the migrated maggots were not recovered.

The first forensic entomological research using pig carcass model in Malaysia was conducted by Heo *et al*. (2007). A three-month-old pig (8.5 kg) died of pneumonia was placed in the field to observe the decomposition stages and the fauna succession of forensically important flies. Heo *et al*. (2007) reported that the first visitor seen within a minute to the pig carcass was muscid fly, followed by ants and spiders; while calliphorid flies were only observed visiting the carcass within half an hour. Heo *et al*. (2007) found that carcass decomposition rate was faster in Malaysia compared to those in Hawaii (Payne, 1965) and Western Australia (Bornemissza, 1957), in which the dry stage was reached on Day 25 and Day 40, respectively. Heo *et al*. (2007) also reported that main flies were *Chrysomya megacephala* (Day 2 to Day 6), but the larvae were mainly those of *Chrysomya rufifacies* (Day 4 to Day 14).

Heo *et al*. (2008a) conducted an insect succession study on a decompositing piglet carcass placed in a man-made freshwater pond in Malaysia. Their study highlighted that there are five stages of decomposition (submerge-fresh, early-floating, floating decay, bloated-deterioration and sunken-remains) of the piglet carcass in the aquatic environment, which naturally occurred in Malaysia, and the majority of flies observed on the floating carcass was *Chrysomya megacephala*, followed by *Chrysomya*

rufifacies, *Ophyra spinigera* and *Musca domestica*. They concluded that insect activities on the floating carcass were not intense compared to carcass placed on the land.

Owing to little published data on the association between burned animal or humans and insect succession, Heo *et al.* (2008b) carried out a study to compare insect successions and rate of decomposition between a partially burned pig carcass and an unburned pig carcass (control) in an oil palm plantation in Tanjong Sepat, Selangor, Malaysia. The results showed that there was no significant difference between the rate of decomposition and sequence of faunal succession on both pig carcasses. The only difference noted was in the number of adult flies, whereby more flies were seen in the control carcasses. Faunal succession on both pig carcasses was in the following sequence: Calliphoridae, Sarcophagidae, Muscidae, Phoridae and Stratiomyidae. Heo *et al*. (2008b) concluded that burning does not affect the rate of decomposition and the sequence of faunal succession. Therefore, it is demonstrated that estimation of post-mortem interval was not affected.

During a forensic entomological study conducted in an oil palm plantation in Tanjong Sepat, Heo *et al*. (2008c) collected 2 adults of *Bengalia emarginata* Malloch (Diptera: Calliphoridae) around the pig carcass. *Bengalia emarginata* has not been recorded from Malaysia until Heo *et al*. (2008c) reported it as a new record in Malaysia. Heo *et al*. (2008c) suggested that forensic entomologist in Malaysia should be more vigilant on the probability of *Bengalia emarginata* having a role in forensic investigation, by virtue that this fly has been found to be attracted to a decomposed animal.

Chen *et al*. (2008a) reported the occurrence of signal fly, *Scholastes* sp. (Diptera: Platystomatidae) landed on the pig carcass in an oil palm plantation in Tanjong Sepat and monkey carcass in forested area in Gombak, within 5 minutes and an hour of placement of carcasses, respectively. Both animal carcasses were visited by

Scholastes sp. during the fresh decomposition period. So far, there was no report on Malaysian species of Platystomatidae playing a forensic role. The role of *Scholastes* flies in the decomposition process remains unknown.

Heo *et al*. (2008d) reported the first record on the occurrence of *Musca domestica* (Diptera: Muscidae) ovipositing on a freshly dead pig in an oil palm plantation in Tanjong Sepat, Selangor. However, no maggots were found in their study.

Zuha *et al*. (2009) reported the first and new record on the occurrence of *Myospila pudica pudica* (Diptera: Muscidae) visiting the monkey carcass in a forested area in Bangi, Selangor. The *Myospila pudica pudica* was found to visit the carcass at fresh-bloating stage and advanced decay stage of decomposition. *Myospila pudica pudica* was a single visitor to the carcass and found on the external surface of the carrion, without swarming and ferocious activities, unlike common forensic calliphorids (Zuha *et al*., 2009).

Azwandi & Abu Hassan (2009) carried out the first study of Diptera associated with the exploitation of carcasses conducted in an oil palm plantation in Bandar Baharu, Kedah during the dry and wet seasons. Their study shows that the duration of the fresh and bloated stages of decay were the same in wet and dry seasons but later stages of decay were markedly shorter during wet season. The percentage of calliphorids abundance in wet season and dry season was 50.83% and 35.20%, respectively. Azwandi & Abu Hassan (2009) also reported that *Chrysomya megacephala* and *Chrysomya nigripes* were recognized as the earliest arrivals on the first day of exposure, and adult *Chrysomya nigripes* was abundant for approximately two weeks after placement of the monkey carcasses.

Chen *et al.* (2010) conducted a study on insect succession of a monkey carcass in a forested area in Ulu Gombak, Selangor. The third-instar larvae of the house fly, *Musca domestica* (Linnaeus) (Diptera: Muscidae) were only found on dry stage of a

decomposed (Day-33) monkey carcass. This observation revealed that *Musca domestica* maggots were able to colonise together with other muscid fly's maggots, *Hydrotaea (=Ophyra) spinegera* on remains stage of a carcass. However, the role of *Musca domestica* on forensic entomological study remains unknown. Since *Musca domestica* larvae have never been found in human corpses (Lee *et al.*, 2004), Chen *et al.* (2010) recorded the first finding of *Musca domestica* maggots on primate carcass in Malaysia.

2.4 STUDIES ON THE DISTRIBUTION AND BIONOMICS OF FORENSICALLY IMPORTANT FLIES IN MALAYSIA

In May 1978, Hanski (1981) carried out a study on distribution of carrion flies in tropical rain forests in Sarawak, Malaysia. He discovered 575 specimens of 22 species of carrion flies from 8 different forest types in Gunung Mulu National Park, Sarawak. According to Hanski (1981), the common species preferred different altitudes and forest types. All *Chrysomya* spp. (*Chrysomya megacephala*, *Chrysomya defixa*, *Chrysomya nigripes*, *Chrysomya pinguis* and *Chrysomya villeneuve*) were found in the lowland forest below 800m, but some preferred the alluvial forest, whereas others occurred more numerously in the mixed dipterocarp forest. Hanski (1981) also reported that *Lucilia porphyrina* (Walk) was the only abundant calliphorid between 800m and 1600m, and 2 *Calliphora* spp. (*Calliphora atripalpis* Mall and *Calliphora fulviceps* Wulp) were confined to the upper montane forests.

In early 2000s, some studies on the distribution and bionomics of calliphorids and muscids were carried out. Omar *et al*. (2003a) studied the distribution and bionomics of different species of Muscidae and Calliphoridae in several locations around Kuala Lumpur and Gombak areas in order to elucidate the synanthropic behaviour of Malaysian flies. They reported that the asynanthropic flies are *Bengalia*

labiata, *Hypopygiopsis violacea*, *Hypopygiopsis fumipennis*, *Chrysomya defixa* and *Chrysomya nigripes*; while hemisynanthropic flies in ascending order of synanthropy are *Chrysomya villeneuve*, *Chrysomya chani*, *Musca sorbens*, *Hemipyrelia ligurriens*, *Musca domestica*, *Chrysomya rufifacies*, *Lucilia cuprina* and *Chrysomya megacephala* (Omar *et al*., 2003a).

Omar *et al*. (2003b) conducted a study to elucidate distribution of Malaysian flies, their food preference and sex ratio at 7 locations of different altitudes along Titiwangsa (Kuala Lumpur, Malaysia) transect, and they collected 32 species of flies belonging to 7 families i.e. Calliphoridae, Muscidae, Anthomyiidae, Sarcophagidae, Lauxaniidae, Otitidae and Tephritidae. They reported that (i) prawns and liver were equally effective in attracting the flies and only a handful of species were attracted to fruits, (ii) female flies dominated in term of numbers at all study sites, and (iii) blow fly density was not a simple inverse relationship in relation to altitude but appears to maximize at intermediated elevation (Omar *et al*., 2003b).

Omar *et al*. (2003c) studied the distribution and bionomic of blow flies at a five-storey building in Kuala Lumpur by using cattle liver as bait. They reported that (i) the blow fly was found at every level of the building, except for the highest floor, (ii) fly density decreased with increasing level of the building, and (iii) nocturnal observation at the ground floor, where the fly density was the highest during day time, did not detect any flies.

In 2007, Nazni *et al*. investigated the distribution and abundance of diurnal and nocturnal dipterous flies using multiple baits in the Federal Territory, Putrajaya, Malaysia. Nazni *et al*. (2007) collected 23 species of flies belonging to 6 families i.e. Calliphoridae, Chrysomyidae, Muscidae, Sarcophagidae, Tachinidae and Ullilidae. Their findings show that *Chrysomya megacephala* was the most dominant species, followed by species of Sarcophagidae and *Musca domestica*. Diurnal period had more

number of flies (81.55%) compared to the nocturnal period (18.45%). Nazni *et al*. (2007) also reported that 9 species of flies (*Sarcophaga misera*, *Musca ventrosa*, *Anthomyia iliocata*, *Dichaetomyia* sp., *Phumosia testacea*, *Cosmina* sp., *Stomorhina discolor*, Ullitidae and Tachanidae) were strictly diurnal, while only 1 species (*Lipse* cf. *leucospila*) was strictly nocturnal.

Zuha *et al*. (2008) conducted a study on analysis on the fly artefacts produced by forensically important blowfly, *Chrysomya megacephala*. Documentation on the characteristics of fly artefacts is essential to interpret these bloodstains because the presumptive test for blood using Saugur and Luminol could not differentiate stains of flies from those of victims (Bevel & Gardner, 2002). Thus, Zuha *et al*. (2008) highlighted the important feature of fecal spots, vomitous spots and swiping stains produced by *Chrysomya megacephala*. This information could be useful for forensic scientists in differentiating blood spatters that originated from the victims and those formed by flies, especially in the process of crime event reconstruction.

Rashid *et al*. (2008) conducted a study to examine the effect of malathion on the development of *Chrysomya megacephala*. Their results indicated that for the first 6 to 30 hours, larvae from the control group developed more rapidly than larvae feeding on tissue containing malathion, and the time required for adult emergence was significantly greater for malathion-treated colony which was 10 days compared to 7 days in control colony. Rashid *et al*. (2008) concluded that the presence of malathion altered the development rate of *Chrysomya megacephala* and thus disrupted normal post-mortem interval estimation.

Precise development data of blow flies, to be used as a forensic indicator, are essential for accurate estimation of pos-mortem interval. Chen *et al*. (2008b) studied the larval growth parameters and growth rates of forensically important species, *Hypopygiopsis violacea* and *Chrysomya rufifacies* at an ambient temperature of 28 ±

2ºC, relative humidity at 70 ± 5% and photo period at 12 h dark : 12 h light. They reported that total development time for *Hypopygiopsis violacea* and *Chrysomya rufifacies* was 308.25 ± 8.25 hours and 227.00 ± 8.35 hours, respectively. On the other hand, the larval growth of another forensically important muscid fly, *Synthesiomyia nudiseta* was studied by Kumara *et al*. (2009b). The study was conducted in a room with ambient temperature of 28.5 ± 1.5ºC, relative humidity within 67 – 85% and photo period at 15 h dark : 9 h light. The total developmental time of *Synthesiomyia nudiseta* took 322 ± 19 hours. Chen *et al*. (2008b) and Kumara *et al*. (2009b) recorded for the first time on the growth characteristics of *Hypopygiopsis violacea* and *Synthesiomyia nudiseta* in this region, respectively.

2.5 KEY TO FORENSICALLY IMPORTANT FLIES AND LARVAE IN MALAYSIA

The first taxonomy key of forensically important flies was published by Cheong *et al*. (1971), entitled *IMR Pictorial Keys for Common Flies*. This pictorial key recorded the common forensically important flies found on human corpses in Malaysia. Few years later, Singh *et al*. (1979) published a key entitled *A Preliminary Key to Common Calliphorid Flies of Peninsular Malaysia*. This key included the illustration of the genitalia of some rare species, list of new localities, altitudes and other ecological data of calliphorids in Peninsular Malaysia. In 1980, *IMR Pictorial Key on Fly Maggots* was published by Mahadevan *et al*. (1980).

In 1997, a key entitled *Blow Flies of Malaysia and Singapore* was published by Kurahashi *et al*. (1997) in *The Raffles Bulletin of Zoology*. The key of Kurahashi *et al*. (1997) reviewed 118 species of adult flies belonging to the family of Calliphoridae from

Malaysia and Singapore, and the list of new localities, distributions and ecological data of all these flies were also added.

Few years later, the *Key to 3rd Instar Larvae of Forensic Importance in Malaysia* (Omar, 2002), *Key to the Calliphorid adults of Forensic Important in the Oriental Region* (Kurahashi, 2002a) and *Key to the Sarcophagid of the Oriental Region* (Kurahashi, 2002b) were published in *Entomology and the Law* by Greenberg & Kunich (2002). The first key recorded the taxonomy features of 9 species of fly larvae commonly found in human corpses in Malaysia, and the 2 later keys recorded the taxonomy features of adult flies of Calliphoridae and Sarcophagidae obtained from forensic entomological studies in Thailand and Malaysia. All these keys provided useful information on larvae and adult flies of forensically importance for researchers in tropical region, to conduct forensic entomological studies in the future.

In 2011, a comprehensive taxonomy key entitled *Illustrated keys to adult flies of forensic importance in Malaysia* was published by Nazni *et al*. (2011).

2.6 STUDIES ON OTHER CARRION INSECTS IN MALAYSIA

The distributional ecology of 66 species of dung and carrion-feeding beetles (Coleoptera: Scarabaeidae) in tropical rain forests in Gunung Mulu National Park, Sarawak was studied by Hanski (1983). Hanski (1983) reported that species richness was highest in the lowland forest, with the exception of the tropical heath forest, where the density of Scarabaeidae was low, apparently because of scarcity of resources. In another study on carrion beetles (Staphylinidae) in tropical rain forest in Sarawak, Hanski & Hammond (1986) collected a total of 16,000 individuals from 9 types of tropical rain forest in Gunung Mulu National Park, including 110 species of Staphylinidae exclusive of the Aleocharinae, 1 species of silphid and 6 species of

Histeridae. Both studies by Hanski (1983) and Hanski & Hammond (1986) reported that species number began to decrease with increasing altitude at 200m above sea level. However, both studies only reported the biodiversity and distributional ecology of carrion beetles (Scarabaeidae and Staphylinidae) in tropical rain forest in Sarawak.

In a forensic entomological study firstly conducted in Tanjong Sepat, Heo *et al*. (2008e) reported the incidence of spider predated on forensically important fly in Malaysia, Heo *et al*. (2008e) reported a spider, *Oxyopes* sp. (Oxyopidae) preying on a blowfly, *Chrysomya rufifacies* by using a pair of chelicerae and injecting venom into the fly. The fly tried to escape from it is clutch but failed. Their study suggested that the behavioral studies on spiders in this country should be emphasized and conducted for a better understanding of the role they may play as predators of flies for forensic importance.

Heo *et al*. (2009) reported for the first time on six species of ants recovered from pig carcasses placed in an oil palm plantation in Tanjong Sepat, Selangor. Ants collected belonged to the family Formicidae and to three subfamilies: Formicinae (*Oecophylla smaragdina* and *Anoplolepis gracilipes*), Myrmicicnae (*Tetramorium* sp. and *Pheidologeton* sp.) and Ponerinae (*Odontoponera* sp. and *Diacamma* sp.). According to Heo *et al*. (2009), ants were typically observed shortly after death and could be found at all stages of decomposition. In addition, these ants predated fly eggs, larvae, pupae and adults, and some were seen feeding on tissues of a pig carcass.

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CHAPTER 3

MATERIALS AND METHODS

3.1 STUDY SITE

3.1.1 GOMBAK (LOWLAND FORESTED AREA)

Four studies (Study 1 – Study 4) on insect succession were conducted from 9 May 2007 to 25 October 2007 to determine successional arthropods using monkey carcasses in Wildlife Research Center, University of Malaya, 16th Mile of Gombak District (3º17’57.86”N, 101º47’00.78”E), Selangor, Malaysia (Figure 3.1). The study site was a secondary lowland forested area (200 meters above sea level) with very low human populations and activities. The nearest human dwelling was approximate 300 meters from the study site.

Figure 3.1. Environmental conditions of study site in lowland forested area located in Ulu Gombak, Selangor.

3.1.2 TANJONG SEPAT (COASTAL AREA)

Four studies (Study 5 – Study 8) on insect succession were conducted from 6 May 2008 to 5 August 2008 to determine successional arthropods using monkey carcasses in Tanjong Sepat (2º39'12.29"N, 101º34'27.66"E), Selangor, Malaysia (Figure 3.2). The study site was a coastal area (6 meters above sea level) with high human populations and activities. The beach was not clean, garbage was found around the study site. Human dwelling was found near by the coastal area.

Figure 3.2. Environmental conditions of study site in coastal area located in Tanjong Sepat, Selangor.

3.1.3 BUKIT CINCIN (MONTANE FORESTED AREA)

Four studies (Study 9 – Study 12) on insect succession were conducted from 26 May 2009 to 9 July 2009 to determine successional arthropods using monkey carcasses in Bukit Cincin (3º26'21.39"N, 101º46'59.36"E), Pahang, Malaysia (Figure 3.3). The study site was a highland area (1,748 meters above sea level) with very low human activities. No human dwelling was found near the study site. The nearest human dwelling was approximately 4km from the study site. The highland was surrounded by primary forest.

Figure 3.3. Environmental conditions of study site in montane forested area located in Bukit Cincin, Pahang.

3.2 INDOOR AND OUTDOOR CONDITIONS FOR FAUNA SUCCESSION STUDY

For comparison purpose, the outdoor and indoor studies were conducted at two different sites located at the same region having similar vegetation. Both sites were separated by a distance of 200 m. The outdoor site was partially shaded with trees and scrubs.

A cemented ground wooden hut [300 cm (length) x 300 cm (wide) x 300 cm (height)] (Figure 3.4 – Figure 3.6) that had 4 windows [120 cm (height) x 60 cm (wide)] on each site of the wall was constructed in each study site. The wooden hut was used to simulate a medium room size in Malaysia.

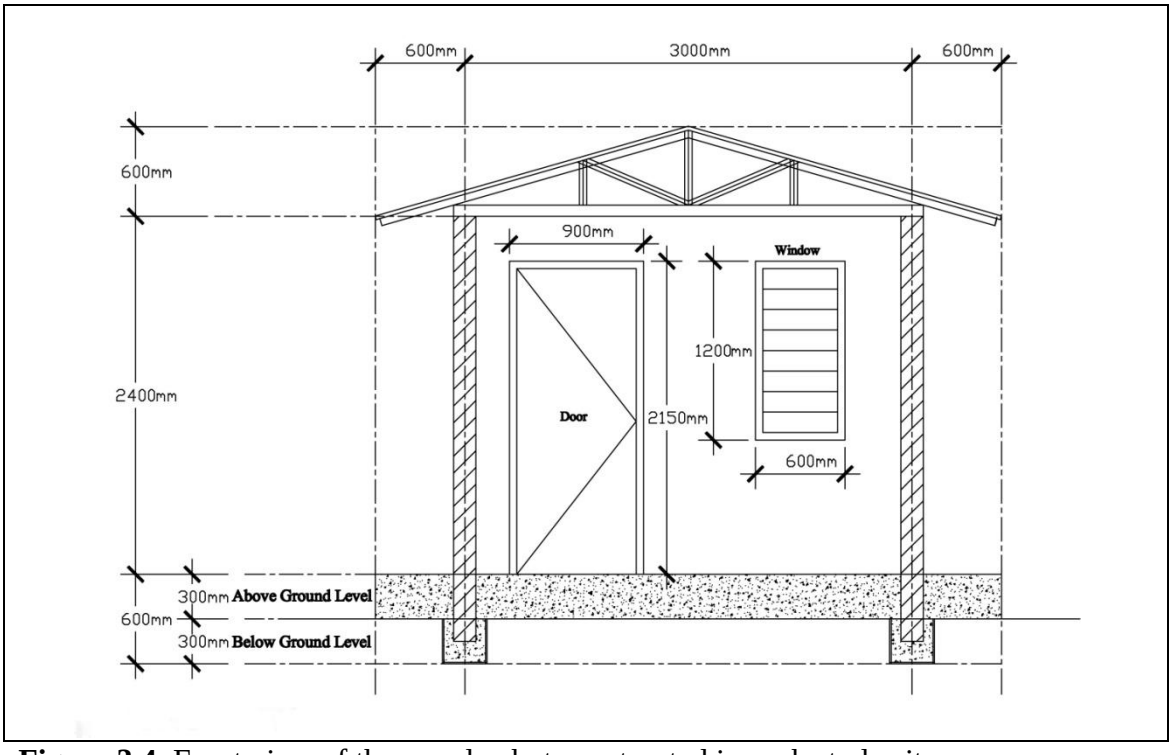

Figure 3.4. Front view of the wooden hut constructed in each study site.

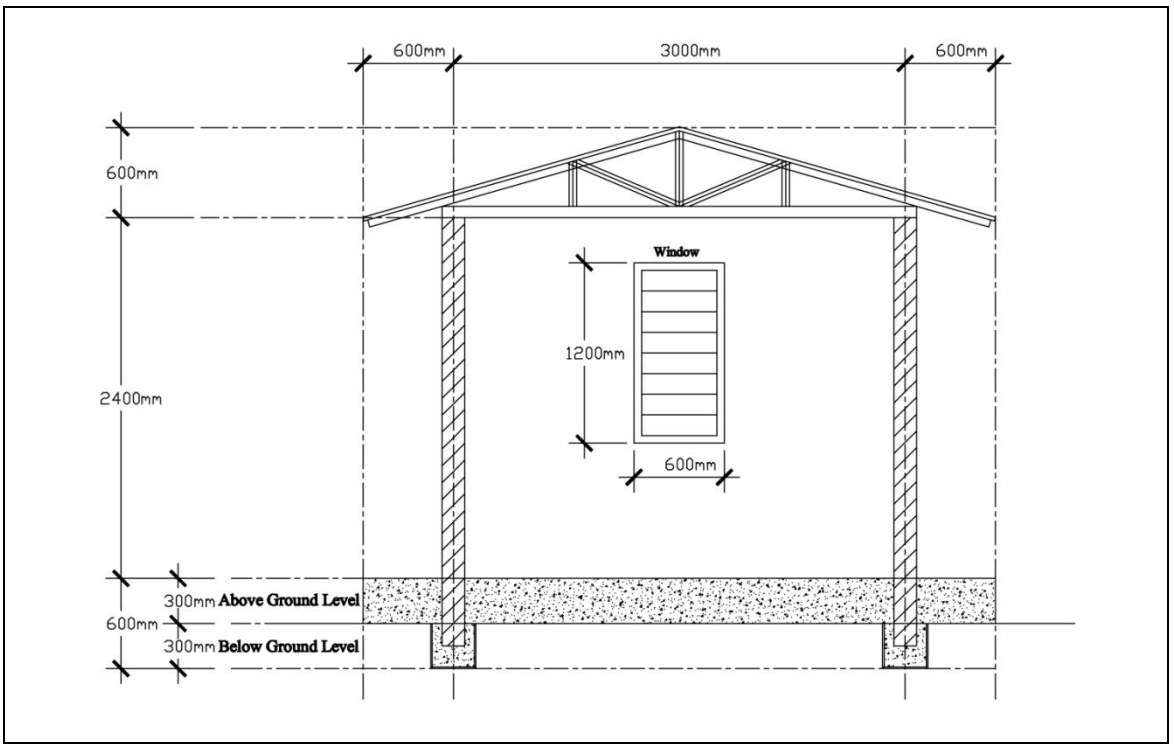

Figure 3.5. Side (left and right) and posterior views of the wooden hut constructed in each study site.

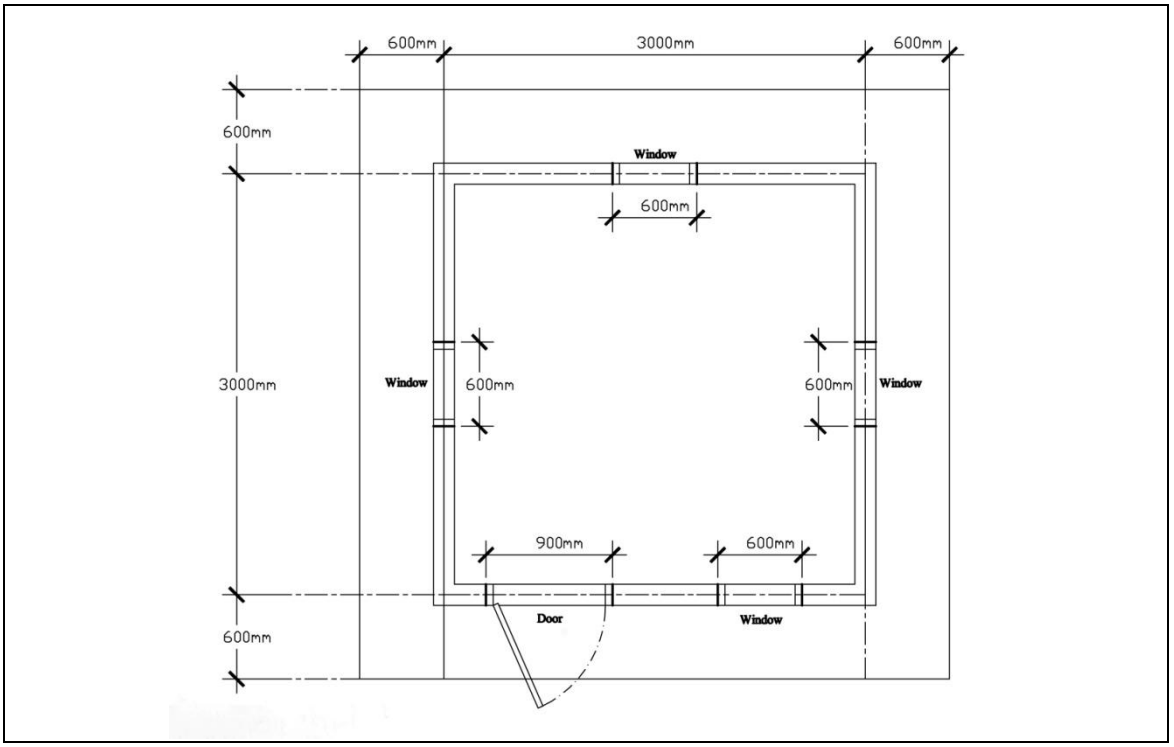

Figure 3.6. Front elevation of the wooden hut constructed in each study site.

3.3 ANIMAL CARCASSES

The monkey (*Macaca fascicularis,* Raffles) carcasses were used as a model for human decomposition since they are phylogenetically related to human. The monkeys were about 3 year olds and their weights ranged from 4.5 kg to 5.6 kg.

3.4 EUTHANIZATION OF MONKEY

At the beginning of field study, the monkeys were euthanised by a single shot to the forehead from a handgun at point blank. Enthanisation was administrated by personnel of the Department of Wildlife and National Parks (PERHILITAN), Peninsular Malaysia, Ministry of Natural Resources and Environment, Malaysia. The study protocol was approved by Institute for Medical Research's Unit of Animal Care and Use Committee [ACUC/KKM/02(2/2008)]. No faeces and urine of the carcasses were observed after the monkeys were euthanised.

3.5 STUDY DESIGN

3.5.1 FAUNA SUSCESSION ON MONKEY CARCASSES PLACED IN OUTDOOR AND INDOOR IN DIFFERENT ECOLOGICAL HABITATS

After death was confirmed, the monkey carcasses were immediately clothed with a white cotton short sleeve t-shirt to simulate human cadavers. One monkey carcass was immediately placed outdoor, shaded under the trees and near the bushes. The carcass was then covered with metal wire mesh (2.5 cm^2) cage measuring 90 cm (length) x 60 cm (width) x 45 cm (height). The cage was bottomless to allow the carcass to lay

directly in contact with the ground. The bottomless cage was fixed to the ground using a 15 cm metal hook at each side of the cage to avoid the cage being lifted by scavengers. Another monkey carcass was placed indoor on a cemented ground of a wooden hut. The windows of the wooden hut were open throughout the study period to allow insects access into the hut. A total of 2 replicates were used in outdoor and indoor, respectively. One set of study (consisted of 1 outdoor study and 1 indoor study) was started during daytime and the monkeys were euthanized at 1130h; while another set (consisted of 1 outdoor study and 1 indoor study) was started after sunset and the monkeys were euthanized at 2200h. This was conducted to observe if there is oviposition of flies during nocturnal period. For comparison purpose, both outdoor and indoor studies were conducted simultaneously.

The monkeys which were euthanized during diurnal period were monitored hourly (10 minutes for each hour) for the first 3 days, and daily from day 4 onwards until only bones were fully exposed and no more larvae were found. Monkeys that were euthanized during nocturnal period were monitored hourly (10 minutes for each hour) throughout the night until the sunrise on the next day and continued to be monitored hourly for the next two days and then daily from day 4 onwards until only bones were fully exposed and no more larvae were found. In all the studies, environmental parameters such as temperature and relative humidity were recorded daily by using a digital thermo-hygrometer. The data of daily rainfall amount were provided by Department of Meteorology Malaysia, which were collected by meteorological station located approximately 5 km from the study site.

Adult insects which visited the monkey carcasses were collected by using an insect sweep net. A representative sample of the total insects from different larval masses was collected so that the natural populations were not disturbed. Larvae were collected by using art-brush and forceps, and immediately placed in to glass vials

containing 70% ethanol. Pupae were also collected and brought to the laboratory for adult emergence.

This study was conducted in three different ecological habitats, namely lowland forested area, coastal area and montane forested area.

3.5.2 FAUNA SUSCESSION ON INCINERATED MONKEY CARCASSES IN LOWLAND FORESTED AREA

After death was confirmed, the monkey carcass was immediately clothed to simulate human cadavers. A total of 3L of diesel was applied onto the monkey carcass evenly. The carcass was then burned to simulate a case of victim incinerated by a murderer to destroy the evidence. In this study, the carcasses were incinerated to give a Crow-Glassman Scale (CGS) at level 2 with 100% body surface / skin burned (Glassman & Crow, 1996). The incineration of monkey carcasses took about 20 minutes and the cooling process took about 15 minutes. The carcasses were considered cool when no smoke was observed and no heat was felt when probed with a hand placed near the carcasses.

The carcass was left exposed on the ground shaded under the trees and covered with a metal wire mesh (2.5 cm^2) cage measuring 90 cm (length) x 60 cm (width) x 45 cm (height). The cage was bottomless to allow the carcass to lay directly in contact with the ground. The bottomless cage was fixed to the ground using a 15 cm metal hook at each side of the cage to avoid the cage being lifted by scavengers.

The observation on insect activities on the carcasses, decomposition process of carcasses and collection on adult and immature insect specimens were done as documented in Section 3.5.1. A total of 2 replicates were used. The environmental parameters such as temperature and relative humidity were also recorded daily by using

a digital thermo-hygrometer. The data of daily rainfall amounts were provided by Department of Meteorology Malaysia, which were collected by meteorological station located approximately 5 km from the study site.

This study was conducted only in lowland forested area.

3.5.3 FAUNA SUSCESSION ON MONKEY CARCASSES SUBMERGED IN FRESHWATER RIVER IN LOWLAND FORESTED AREA

After death was confirmed, the monkey carcass was immediately clothed to simulate human cadavers. The carcass was then kept inside a metal cage and placed into the river with running water to simulate a case of victim submerged by a murderer to destroy the evidence. The cage measured 45 cm (length) x 45 cm (width) x 45 cm (height) and consisted of 5 cm x 5 cm wire to allow the accessible of insects fauna.

The observation on insect activities on the carcasses, decomposition process of carcasses and collection on adult and immature insect specimens were done as documented in Section 3.5.1. A total of 2 replicates were used. The environmental parameters such as temperature and relative humidity were also recorded daily by using digital thermo hygrometer. The data of daily rainfall amount were provided by Department of Meteorology Malaysia, which were collected by meteorological station located approximately 5 km from the study site.

This study was conducted only in lowland forested area.

3.5.4 FAUNA SUSCESSION ON MONKEY CARCASSES TREATED WITH INSECTICIDE IN LOWLAND FORESTED AREA

After death was confirmed, the monkey carcass was immediately clothed to simulate human cadavers. A total of 300 mL of malathion (technical grade, 96.6% w/w) was diluted with 1 L water and applied onto the monkey carcasses evenly. The carcass was left exposed on the ground shaded under the trees and covered with a metal wire mesh (2.5 cm^2) cage measuring 90 cm (length) x 60 cm (width) x 45 cm (height). The cage was bottomless to allow the carcass to lay directly in contact with the ground. The bottomless cage was fixed to the ground using a 15 cm metal hook at each side of the cage to avoid the cage being lifted by scavengers.

The observation on insect activities on the carcasses, decomposition process of carcasses and collection on adult and immature insect specimens were done as documented in Section 3.5.1. A total of 2 replicates were used. The environmental parameters such as temperature and relative humidity were also recorded daily by using a digital thermo-hygrometer. The data of daily rainfall amount were provided by Department of Meteorology Malaysia, which were collected by meteorological station located approximately 5 km from the study site.

This study was conducted only in lowland forested area.

3.6 MOUNTING FLY LARVAE

The fly larvae were mounted according to the method described by Lee *et al*. (1984). Larvae were first washed in distilled water, and then cleared in 10% KOH solution for 2 – 6 hours. The larvae were washed again in distilled water, transferred to 10% acetic acid for 30 minutes and washed again in distilled water. At this stage, all the internal

organs of the larvae were removed and the posterior spiracles were cut transversely. The specimens were then dehydrated in ascending series of ethanol (30%, 50%, 70% and 90%) for 30 minutes each. The larvae were then soaked in absolute alcohol for at least 1 hour, and then transferred into clove oil. The specimens were mounted onto a glass slide using Canada Balsam and left in incubator at 40˚C for 1 – 2 days. The slide was examined under a light microscope for taxonomic studies and identification in order to determine the post mortem interval (PMI).

3.7 IDENTIFICATION OF SPECIMENS

The collected specimens were then processed for species identification. Identification of calliphorids were based on the keys of Kurahashi *et al*. (1997) and Greenberg & Kunich (2002) for adults; and Ishijima (1967) for larvae. Identification of muscids was based on the key of Tumrasvin & Shinonaga (1982) and that of platystomatids was based on the key of McAlpine (2001).

Ants were identified by using the key of Bolton (1994) and Holldobler & Wilson (1990). The beetle specimens were identified by Dr. Hans Huijbregts from National Museum of Natural History, Leiden, Netherland and Dr. Munetoshi Maruyama from The Kyushu University Museum, Japan.

3.8 DATA ANALYSIS

Paired t-test and one way ANOVA at a significance level of $p = 0.05$ were used to determine the significant difference between the meteorological data obtained from each study site.

The daily percentage of biomass remaining of monkey carcass from this study was analysed as follow:

$$\text{Percentage of biomass remaining} = \frac{\text{Remaining weight of monkey carcass}}{\text{Weight of monkey carcass at the beginning of study}} \times 100\%$$

3.9 REFERENCES

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CHAPTER 4

RESULTS

The schematic diagram of results reported in this chapter is shown in Figure 4.1. This chapter consists of 13 sections.

At the beginning, this chapter recorded the results obtained from 12 forensic entomological studies conducted in lowland forested area (Gombak, Selangor) (Section 4.1), coastal area (Tanjong Sepat, Selangor) (Section 4.2) and montane forested area (Bukit Cincin, Pahang) (Section 4.3). Each study site consisted of 2 studies which were conducted in outdoor and indoor conditions, respectively.

According to the 24 hours observation on fly activities on monkey carcasses in forested area, the circadian activity pattern of blow flies on monkey carcasses was reported in Section 4.4.

The PMI estimation, and comparison on fly succession and carcarss decomposition process in outdoor and indoor conditions in different ecological habitats were reported in Section 4.5 and 4.6, respectively.

This study also reported the occurence of signal fly, *Scholastes* sp. (Section 4.7) and larvae of house fly, *Musca domestica* (Section 4.8) on forensic entomological study conducted in Malaysia.

Besides these flies (Diptera), the discovery of other forensically important insects such as ants (Hemenoptera) and beetles (Coleoptera) was reported in Section 4.9 and 4.10, respectively.

In order to obtain more useful information regarding insect succession on incinerated human remains, this study simulated a situation in which victim had been deliberately burned to conceal the crime, or those in which cremation has resulted from

an accident or suicide. Thus, the fauna succession and decomposition rate of incinerated monkey carcasses were reported in Section 4.11.

The knowledge on the decomposition rate and fauna succession in different types of aquatic habitats and water body in this region still remains unknown. Section 4.12 documented the fauna succession and decomposition rate of monkey carcasses submerged in freshwater river located in lowland forested area in Malaysia.

In some cases, the murderer may attempt to confuse the evidence by treating the cadaver with a chemical that deters the arrival of forensically important insects such as blow flies for laying eggs. The chemical on the carrion may be absorbed into the body and influenced maggot development. Results in Section 4.13 documented the effect of malathion, an organophosphate on the PMI and succession of insect on monkey carcasses in lowland forested area in Malaysia.

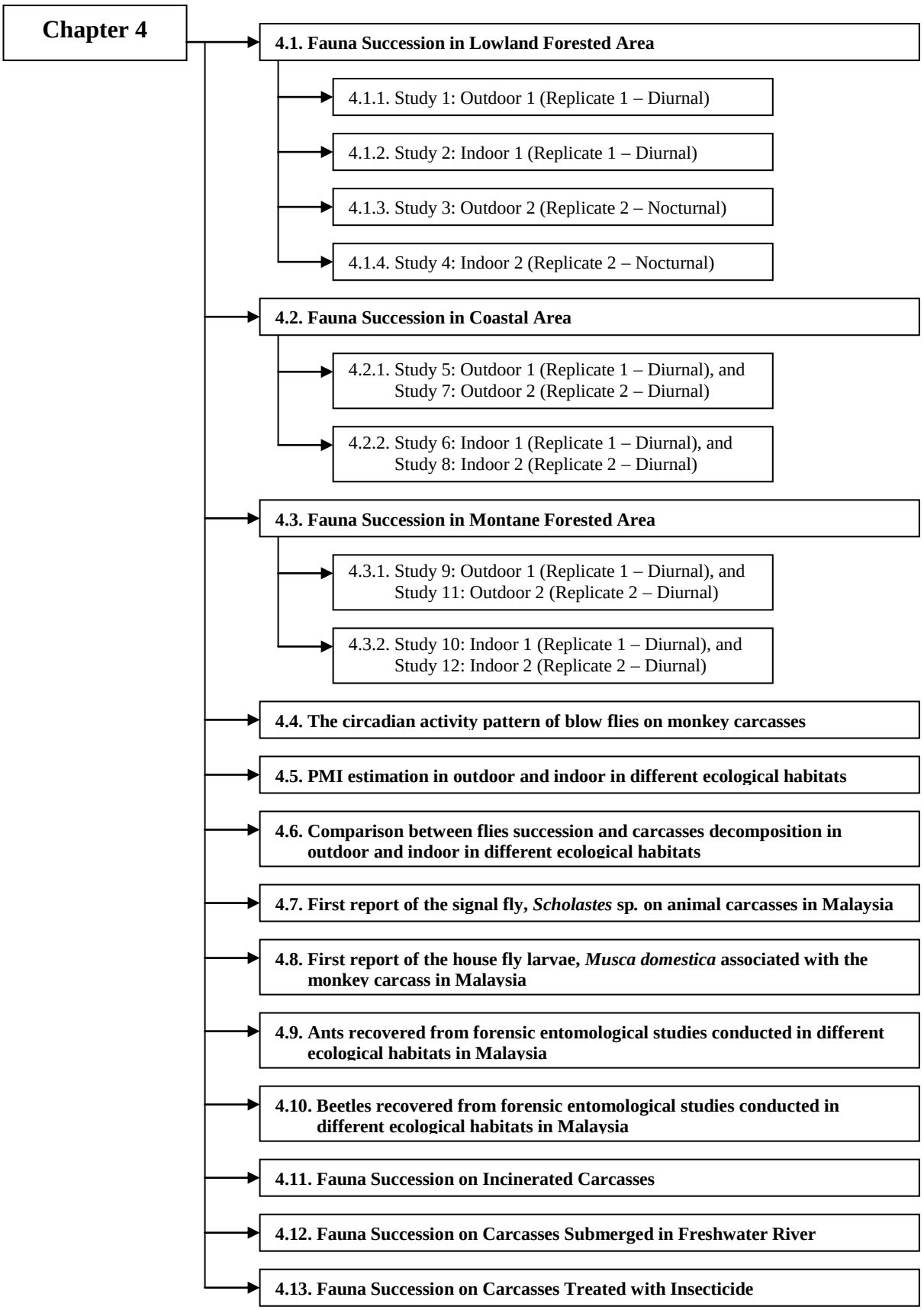

Figure 4.1. Schematic diagram of results consisted in Chapter 4. All results were described in details in respective section.

4.1 ULU GOMBAK (LOWLAND FORESTED AREA)

A total of four studies were conducted in lowland forest located in Ulu Gombak, Selangor. Study 1 was designed to investigate the decomposition process and arthropod succession on monkey carcass placed in outdoor (control); and for comparison purpose, Study 2 was designed to investigate the decomposition process and arthropod succession on monkey carcass placed in indoor. Both studies were commenced daytime from 9 May 2007 at 1130h until 18 June 2007.

Furthermore, Study 3 and Study 4 were conducted to investigate the presence of arthropod activities of especially flies on monkey carcasses in outdoor and indoor conditions at nighttime, respectively. Both studies were commenced on nighttime from 1 August 2007, 2200h until 25 October 2007.

4.1.1 STUDY 1

DECOMPOSITION PROCESS AND ARTHROPOD SUCCESSION ON MONKEY CARCASS IN OUTDOOR (DIURNAL)

Study 1 commenced on 9 May 2007 at 1130h and completed on 23 May 2007. The ambient temperature, relative humidity and daily rainfall amount during the Study 1 are shown in Figure 4.2 and Figure 4.3, respectively. The ambient temperature ranged from 24.9 to 29.7ºC (mean 26.61 ± 0.30ºC) and relative humidity ranged from 71 to 89% (mean 80.17 ± 1.01%). According to the Department of Meteorology Malaysia, 11 rainy days was recorded within 15 days of study period that accounted daily rainfall amount ranged from 1.1 to 28.2 mm (mean 8.72 ± 2.67 mm).

The decomposition of carcass in Study 1 had undergone 5 stages, namely, fresh (Day 1 – 2), bloating (Day 3), decay (Day 4 – 5), advanced decay (Day 6 – 7) and

remains (Day 8 – 15). The percentage of biomass remaining of monkey carcass is shown in Figure 4.4. The decomposition process caused decrease of body weight, leaving only 44.00% of total body weight in the end of this study. The entomofauna obtained from monkey carcass in Study 1 is shown in Table 4.1.

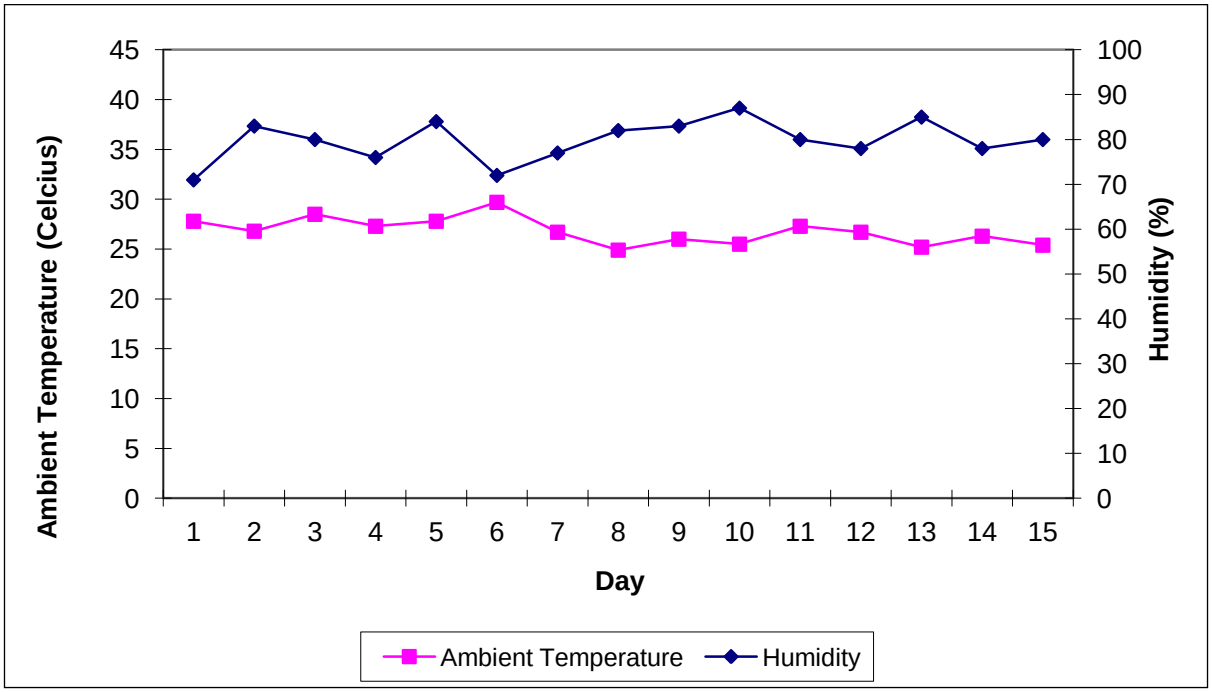

Figure 4.2. Ambient temperature and humidity of the study site for Study 1 conducted in Ulu Gombak, Selangor from 9 – 23 May 2007 (15 Days).

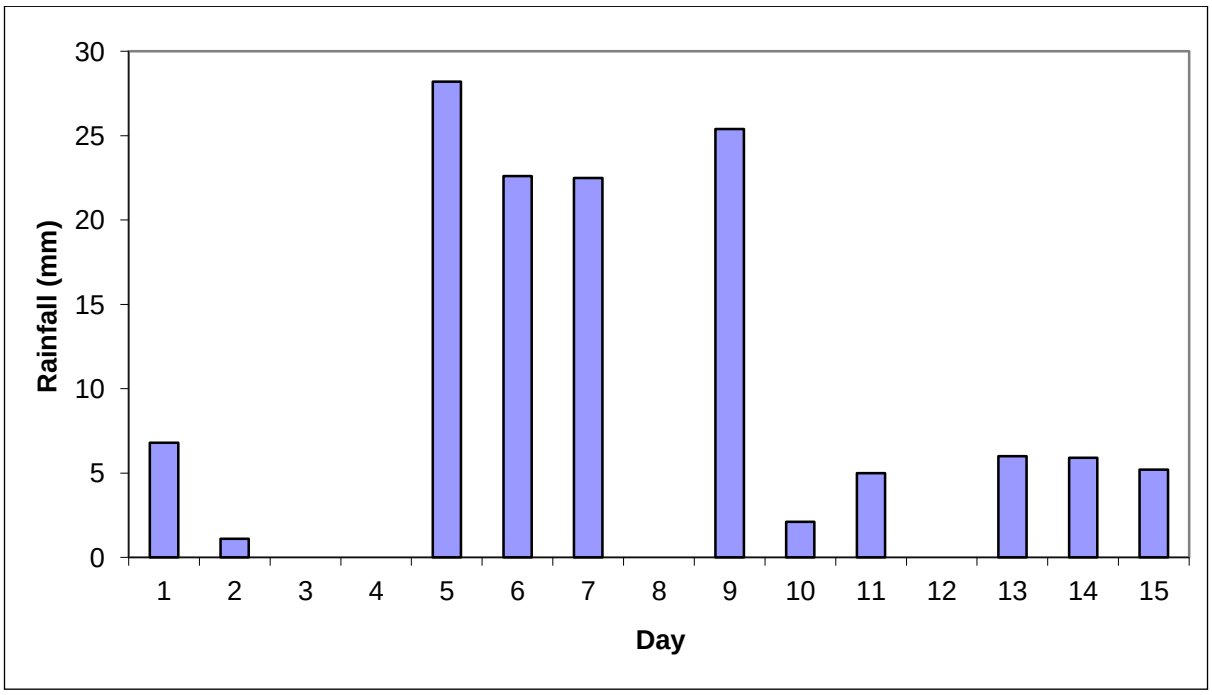

Figure 4.3. Daily rainfall amount in Ulu Gombak, Selangor during Study 1 conducted from 9 – 23 May 2007 (15 Days). Data was provided by the Department of Meteorology Malaysia.

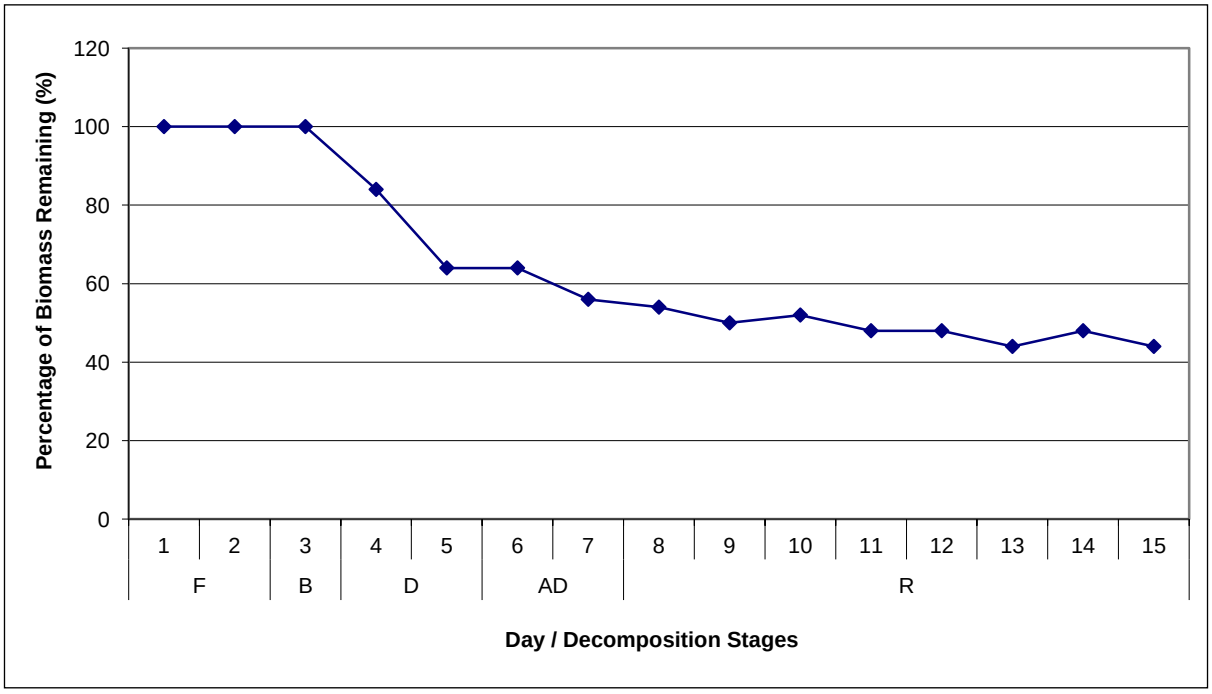

Figure 4.4. Percentage of biomass remaining of monkey carcass in Study 1 placed in lowland forested area located in Ulu Gombak, Selangor. The decomposition of carcass in Study 1 had undergone 5 stages namely, fresh (F), bloating (B), decay (D), advanced decay (AD) and remains (R).

Fresh stage (Day 1 – 2). Five minutes (1145h) after the monkey carcass was placed at the site, the ants [*Pheidologeton diversus* (Hymenoptera: Formicidae: Mymicicnae) and *Odontoponera transversa* (Hymenoptera: Formicidae: Ponerinae)] were attracted to the bloodstain. Within the following 30 minutes (1210h), *Hypopygiopsis violacea* was the first blow fly visiting the carcass, and followed by *Chrysomya megacephala*, *Chrysomya pinguis*, *Scholastes* sp. and *Lucilia* sp. *Hypopygiopsis violacea* first oviposited their eggs in the mouth cavity (1st oviposition site) after 1 hour (1315h) on their visit to the carcass. Within 1 hour later (1355h), many *Chrysomya* spp. began ovipositing their eggs in the ear (2nd oviposition at 1430h) and around the neck region (3rd oviposition at 1640h). Within 6 hours after death (1705h), the eggs oviposited in the mouth cavity hatched and 1st-instar larvae of *H. violacea* were obtained. Flies were observed laying eggs actively throughout the afternoon (1400h till 1900h) on the face, neck and hand regions. Many ants (*Pheidologeton diversus* and *Odontoponera transversa*) were observed preying the fly eggs actively. Flies were observed to leave the carcass during sunset or during twilight zone period. No fly activities were observed after 1925h (sunset time on 9 May 2007 was 1902h) and throughout the night. This phenomenon was observed for three consecutive days. On Day-2, the 2nd-instar larvae of *H. violacea* and *C. pinguis* were collected. The fresh stage lasted for 2 days and no decomposition odour was noticed.

Bloating stage (Day 3). Bloating stage was noticed once the abdomen began to bloat due to accumulated gases produced by bacteria within the intestine or stomach. The larvae of *Chrysomya villeneuve* (2nd-instar), *C. megacephala* (2nd- and 3rd-instar) and *C. pinguis* (3rd-instar) were collected. The bloating stage ended when a small hole was detected at the abdomen when the skin and flesh at the abdomen region were consumed by masses of fly larvae.

Decay stage (Day 4 – 5). Decay stage began when the stomach contents were exposed and the abdomen deflated. In addition, the flesh became creamy and dissociation of body hairs were observed and strong odour was present. Masses of larvae were found on the head, abdomen and anus regions. The percentage of biomass remaining decreased rapidly. Third-instar larvae of *C. villeneuve* and *C. pinguis* were obtained in this stage. The adult beetles, *Phaeochroops rattus* (Coleoptera: Hybosoridae) were collected on Day 5. The decay stage lasted for 2 days.

Advanced decay stage (Day 6 – 7). This stage was noticed when majority of the bones and cartilages were observed. Most of the flesh, inner organs and tissues were consumed by fly larvae. The odour of decaying materials begun to fade. The percentage of biomass remaining decreased slower than the previous stage due to reduced larval activities on the carcass. Very few adult flies (< 10 flies) were observed visiting the carcass. Larvae of *Chrysomya chani* (2nd-instar) and *C. villeneuve* (3nd-instar) were obtained in this stage. Many pupae of *C. villeneuve* were found pupating under the carcass on Day 6. Many ants (*Pheidologeton diversus* and *Odontoponera transversa*) were observed carrying away the fly larvae and pupae. A few carrion beetles, *P. rattus* (Coleoptera: Hybosoridae) and *Onthophagus* nr. *babirussa* (Coleoptera: Scarabaeidae) were found on the carcass. The advanced decay stage lasted for 2 days.

Remains stage (Day 8 – 15). The remains of the carcass were bones, cartilages and some skin layers. Less odour was noticed at this stage. Percentage of biomass remaining decreased very slowly until it stabilised. Third-instar larvae of *C. villeneune*, *C. chani* and *Ophyra spinigera* were collected in this stage. *Chrysomya villeneuve* was the only larvae collected until the end of this study. Newly emerged adult flies (*C. villeneune* and *C. chani*) were noticed from Day 11 onwards. The newly emerged adult flies were seen resting on the brushes near the carcass. Some of the newly emerged adult flies rested on the carcass and were seen predated by the ants. Scarab beetles

(*Onthophagus* nr. *babirussa* and *Onthophagus rudis*) and Staphylinid beetles (unidentified species) were found throughout the remains stage. No larvae were observed after Day 15. The study was terminated on Day 16 when no larvae were found on the carcass.

Table 4.1. Entomofauna attracted to various decomposition stages of monkey carcass (Study 1) placed in outdoor of lowland forested area located in Ulu Gombak, Selangor.

Decomposition stages (Duration, Day)	Order	Family	Species	Stage
Fresh (Day 1 – 2)	Diptera	Calliphoridae	*Hypopygiopsis violacea*	A, E, L1, L2
			Chrysomya megacephala	A, E,
			Chrysomya pinguis	A, E, L1, L2
			Chrysomya villeneuve	A
			Lucilia sp.	A
		Platystomatidae	*Scholastes sp.*	A
		Phoridae	*unidentified sp.*	A
	Hymenoptera	Formicidae	*Odontoponera transversa*	A
			Pheidologeton diversus	A
Bloating (Day 3)	Diptera	Calliphoridae	*Chrysomya megacephala*	A, L2, L3
			Chrysomya pinguis	L3
			Chrysomya villeneuve	A, L2, L3
		Phoridae	*unidentified sp.*	A
	Hymenoptera	Formicidae	*Odontoponera transversa*	A
			Pheidologeton diversus	A
Decay (Day 4 – 5)	Diptera	Calliphoridae	*Chrysomya megacephala*	A
			Chrysomya pinguis	L3
			Chrysomya villeneuve	A, L3
			Chrysomya chani	A
		Phoridae	*unidentified sp.*	A
	Hymenoptera	Formicidae	*Odontoponera transversa*	A
			Pheidologeton diversus	A
	Coleoptera	Hybosoridae	*Phaeochroops rattus*	A
Advanced Decay (Day 6 – 7)	Diptera	Calliphoridae	*Chrysomya megacephala*	A
			Chrysomya villeneuve	A, L3, P
			Chrysomya chani	A, L2
		Muscidae	*Ophyra spinigera*	A
		Phoridae	*unidentified sp.*	A
	Hymenoptera	Formicidae	*Odontoponera transversa*	A
			Pheidologeton diversus	A
	Coleoptera	Hybosoridae	*Phaeochroops rattus*	A
		Scarabaeidae	*Onthophagus nr. babirussa*	A
Dry Remains (Day 8 – 15)	Diptera	Calliphoridae	*Chrysomya villeneuve*	L3, P, NE
			Chrysomya chani	L3, P, NE
		Muscidae	*Ophyra spinigera*	L2, L3
		Phoridae	*unidentified sp.*	A
	Hymenoptera	Formicidae	*Odontoponera transversa*	A
			Pheidologeton diversus	A
	Coleoptera	Scarabaeidae	*Onthophagus nr. babirussa*	A
			Onthophagus rudis	A
		Staphylinidae	*unidentified sp.*	A

A = adult; L1 = 1st instar larvae; L2 = 2nd instar larvae; L3 = 3rd instar larvae; P = pupae; NE = newly emerged adult

4.1.2 STUDY 2

DECOMPOSITION PROCESS AND ARTHROPOD SUCCESSION ON MONKEY CARCASS IN INDOOR (DIURNAL)

Study 2 commenced simultaneously with Study 1 (serve as control) on 9 May 2007 at 1130h and completed on 18 June 2007. The indoor ambient temperature, relative humidity and daily rainfall amount during Study 2 are shown in Figure 4.5 and Figure 4.6, respectively. The indoor ambient temperature ranged from 24.2 to 30.4ºC (mean 26.20 ± 0.18ºC) and relative humidity ranged from 66 to 85% (mean 75.10 ± 0.69%). According to the Department of Meteorology Malaysia, 32 rainy days was recorded within 41 days of study period that accounted daily rainfall amount ranged from 0.2 to 71.5 mm (mean 13.02 ± 2.57 mm).

The decomposition of carcass in Study 2 had undergone 5 stages, namely, fresh (Day 1 – 2), bloating (Day 3 – 6), decay (Day 7 – 8), advanced decay (Day 9 – 20) and remains (Day 21 – 41). The percentage of biomass remaining of monkey carcass is shown in Figure 4.7. The decomposition process caused decrease of body weight, leaving only 22.22% of total body weight at the end of this study. The entomofauna obtained from monkey carcass in Study 2 is shown in Table 4.2.

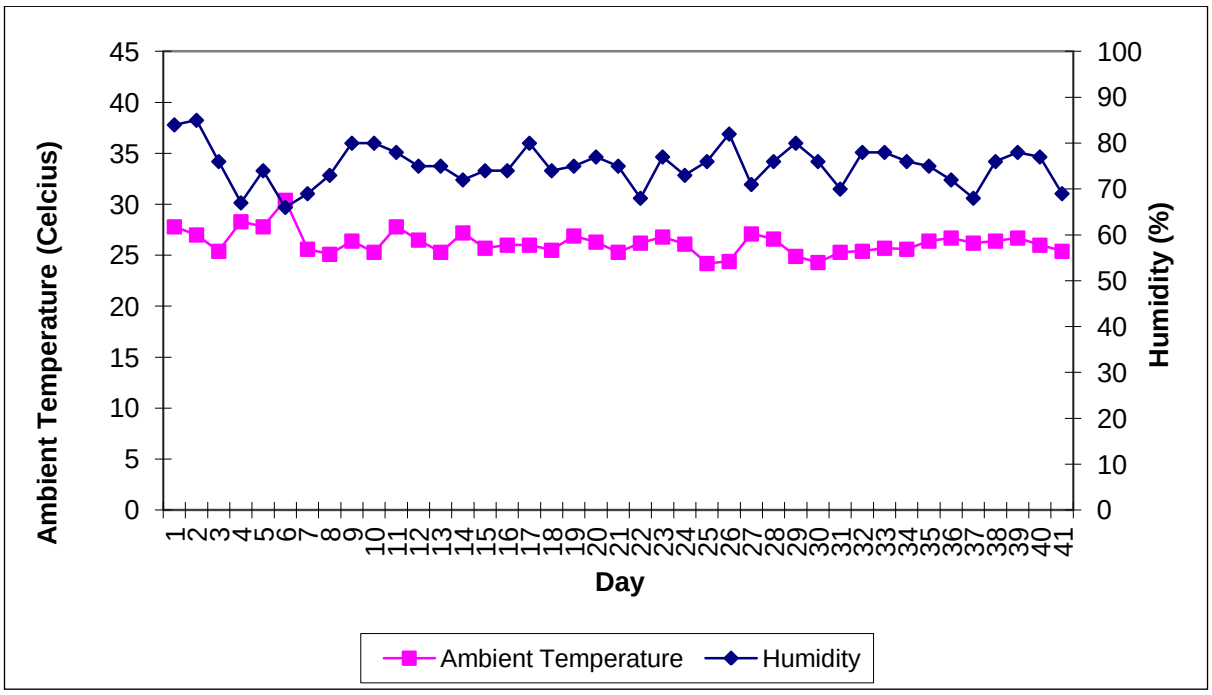

Figure 4.5. Indoor ambient temperature and humidity in the wooden hut during Study 2 conducted in Ulu Gombak, Selangor from 9 May 2007 – 18 June 2007 (41 Days).

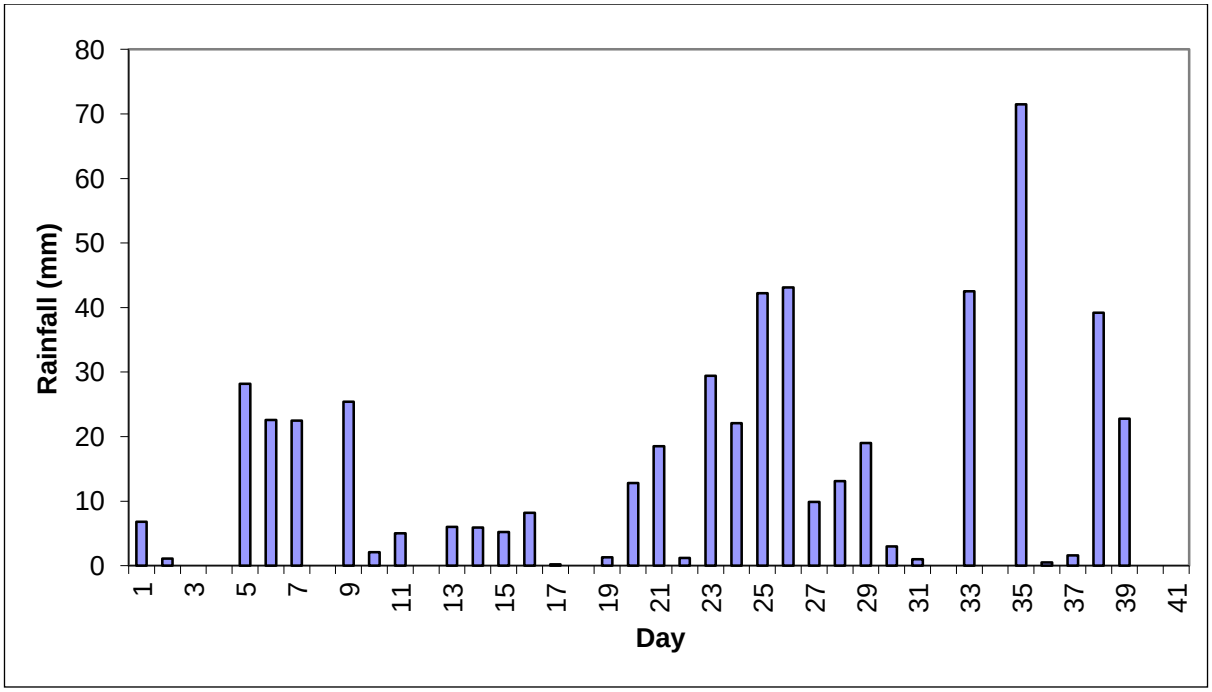

Figure 4.6. Daily rainfall amount in Ulu Gombak, Selangor during Study 2 conducted from 9 May 2007 – 18 June 2007 (41 Days). Data was provided by the Department of Meteorology Malaysia.

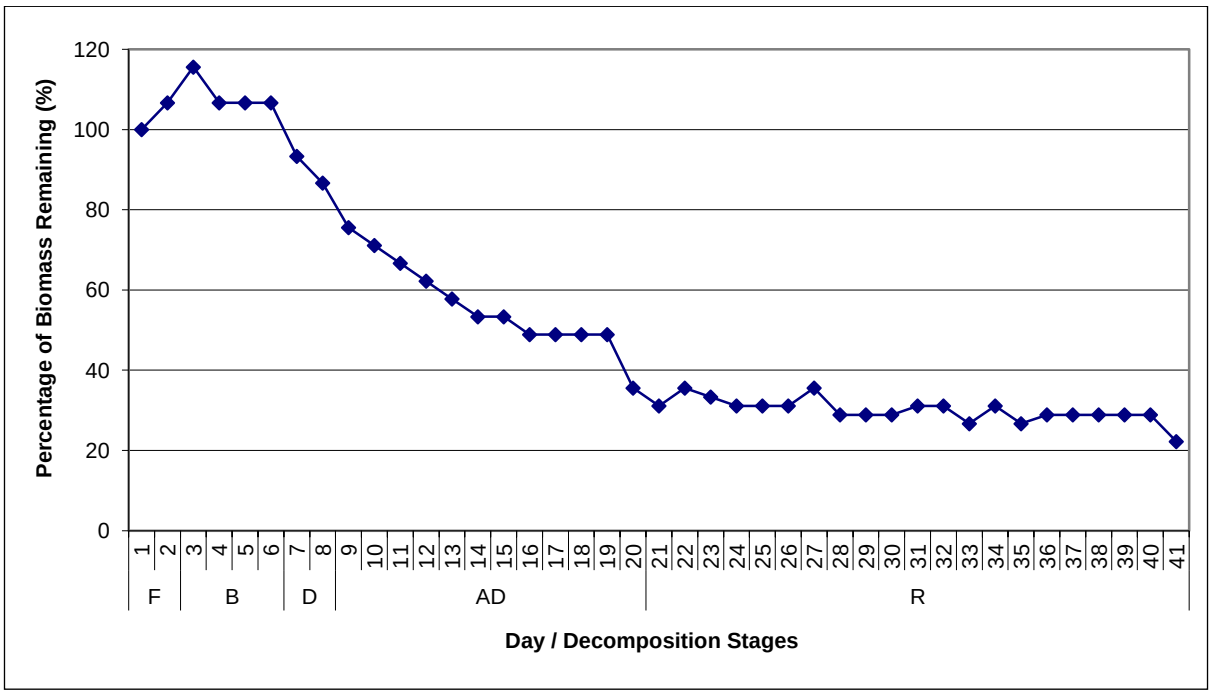

Figure 4.7. Percentage of biomass remaining of monkey carcass in Study 2 placed in lowland forested area located in Ulu Gombak, Selangor. The decomposition of carcass in Study 2 had undergone 5 stages namely, fresh (F), bloating (B), decay (D), advanced decay (AD) and remains (R).

Fresh stage (Day 1 – 2). After euthanising the monkey at 1130h, two species of ants [*Pheidologeton diversus* (Hymenoptera: Formicidae: Mymicicnae) and *Odontoponera transversa* (Hymenoptera: Formicidae: Ponerinae)] were observed as the first arthropods to visit the carcass placed indoor at 1237h. The ants were attracted to the bloodstain on the floor and from the wound on the head throughout this stage. No fly visited the carcass and no oviposition occurred on the carcass during the fresh stage. Percentage of biomass remaining was increased up to 15.57% because no fly larvae infestation on the carcass and the gases produced by the bacteria within the intestine or stomach were trapped in the abdomen. This phenomenon remained until the end of the bloating stage.

Bloating stage (Day 3 – 6). The abdominal bloating was observed on Day 3, but no odour was present. No fly was present in the wooden hut, and no egg mass was observed on the carcass. On Day 4, adults of *Chrysomya villeneuve* and *Chrysomya*

chani (less than 10 flies) were observed visiting the carcass, and egg masses were observed on the eyes and mouth regions of the carcass. However, no larvae were found on Day 4. On Day 5 and Day 6, the entire carcass bloated, slight odour was detected and many flies were found inside the wooden hut (more than 30 flies). Flies were observed around the carcass, and rested on wall and ceiling of the hut. The 2nd-instar larvae of *Chrysomya pinguis*, *C. villeneuve* and *C. chani* were collected from the head and armpit region of the carcass. Adult beetles of *Platydracus* sp. (Coleoptera: Staphylinidae) and *Chrysosilpha formosa* (Coleoptera: Silphidae) were recovered from the carcass. Ants (*Pheidologeton diversus* and *Odontoponera transversa*) were observed actively predating the fly eggs and larvae. The bloating stage lasted for 4 days.

Decay stage (Day 7 – 8). On Day 7, the carcass had deflated and a strong putrefactive odour was present. Hair loss began. Masses of 3rd-instar larvae of *C. pinguis*, *C. villeneuve* and *C. chani* were feeding on the head, armpit, chest and anus regions of the carcass. Many calliphorids (*Chrysomya* sp.) and muscids (*Ophyra spinigera*) were found inside the wooden hut (more than 50 flies). On Day 8, 3rd-instar larvae of *Ophyra spinigera* were present. The carcass was in the rapid state of decay and biomass had reduced to 75.57% by weight at the end of decay stage. Three species of adult coleopteran [*Platydracus* sp. (Staphylinidae), *Chrysosilpha formosa* (Silphidae) and *Phaeochrous emarginatus* (Hybosoridae)] and two species of ants (*Pheidologeton diversus* and *Odontoponera transversa*) were also recovered from the carcass.

Advanced decay stage (Day 9 – 20). On Day 9, the body hairs of the carcass were totally lost. Third-instar larvae of *C. pinguis*, *C. villeneuve*, *C. chani*, *O. spinigera* and Sarcophagidae were actively feeding within the thoracic and abdomen regions. The number of adult calliphorids and muscids present inside the hut reduced (approximately 20 flies), in comparison to the previous stage. On Day 10, post feeding larvae were seen migrating away from the carcass and pupae of *C. villeneuve*, *C. chani* and *C. pinguis*

were collected under the carcass and around the corners of the hut, approximately 120 cm away from the carcass. On Day 13, no fly was observed inside the hut, only adult beetles [*Phaeochrous emarginatus* (Hybosoridae), *Phaeochroops peninsularis* (Hybosoridae), *Aleochara* spp. (Staphylinidae), *Chrysosilpha formosa* (Silphidae) and *Sphaeridium* (Hydrophilidae)] and ants (*Pheidologeton diversus* and *Odontoponera transversa*) were observed predating the larvae and pupae on and around the carcass. Newly emerged adults of *C. villeneuve*, *C. chani* and *C. pinguis* were seen resting inside the hut from Day 16 onwards. Most of the flesh was removed at this stage; however, the decomposition process in this stage was very slow (duration of 12 days). The biomass was reduced to 31.11% by weight at the end of advanced decay stage.

Remains stage (Day 21 – 41). The remains stage begun from Day 21 when most of the bones were observed and remaining flesh and skin of the carcass mummified. *Ophyra spinigera* larvae were found as a dominant coloniser in remain stage, and were abundantly found under the carcass and observed feeding on remaining flesh on the bones. Ants (*Tetramorium* sp.) were observed preying on the *O. spinigera* larvae until end of this study. On Day 22, newly emerged adults of *O. spinigera* were present. The 3rd-instar larvae of *Musca domestica* (Linnaeus) were only found on Day 33 of a decomposed monkey carcass and no *M. domestica* larvae were obtained after this. Beetles belonging to the family of Hybosoridae (*Phaeochroops rattus*), Silphidae (*Chrysosilpha formosa*), Staphylinidae (*Aleochara* sp.) and Lampyridae (unidentified species) were recovered in this stage. The remain stage lasted for 21 days. This study was completed on Day 41, when there was no larvae found on the carcass on the following day.

Table 4.2. Entomofauna attracted to various decomposition stages of monkey carcass (Study 2) placed in indoor of lowland forested area located in Ulu Gombak, Selangor.

Decomposition stages (Duration, Day)	Order	Family	Species	Stage
Fresh (Day 1 – 2)	Hymenoptera	Formicidae	*Odontoponera transversa*	A
			Pheidologeton diversus	A
Bloating (Day 3 – 6)	Diptera	Calliphoridae	*Chrysomya chani*	A, E, L2
			Chrysomya pinguis	L2
			Chrysomya villeneuve	A, E, L2
		Muscidae	*Ophyra spinigera*	A
		Phoridae	*unidentified sp.*	A
	Hymenoptera	Formicidae	*Odontoponera transversa*	A
			Pheidologeton diversus	A
	Coleoptera	Silphidae	*Chrysosilpha formosa*	A
		Staphylinidae	*Platydracus sp.*	A
Decay (Day 7 – 8)	Diptera	Calliphoridae	*Chrysomya chani*	A, L3
			Chrysomya megacephala	A
			Chrysomya pinguis	L2, L3
			Chrysomya villeneuve	A, L3
		Muscidae	*Ophyra spinigera*	A, L3
		Phoridae	*unidentified sp.*	A
	Hymenoptera	Formicidae	*Odontoponera transversa*	A
			Pheidologeton diversus	A
	Coleoptera	Hybosoridae	*Phaeochrous emarginatus*	A
		Silphidae	*Chrysosilpha formosa*	A
		Staphylinidae	*Platydracus sp.*	A
Advanced Decay (Day 9 – 20)	Diptera	Calliphoridae	*Chrysomya chani*	A, L3, P, NE
			Chrysomya megacephala	A
			Chrysomya pinguis	L3, P, NE
			Chrysomya villeneuve	A, L3, P, NE
		Muscidae	*Ophyra spinigera*	A, L3, P
		Phoridae	*unidentified sp.*	A
		Sarcophagidae	*unidentified sp.*	L3
	Hymenoptera	Formicidae	*Odontoponera transversa*	A
			Pheidologeton diversus	A
	Coleoptera	Hybosoridae	*Phaeochroops peninsularis*	A
			Phaeochrous emarginatus	A
		Hydrophilidae	*Sphaeridium sp.*	A
		Silphidae	*Chrysosilpha formosa*	A
		Staphylinidae	*Aleochara sp.*	A
			unidentified sp.	A
Dry Remains (Day 21 – 41)	Diptera	Muscidae	*Musca domestica*	L3
			Ophyra spinigera	A, L3, P, NE
		Phoridae	*Phorid sp.*	A
	Hymenoptera	Formicidae	*Tetramorium sp.*	A
			Pheidologeton diversus	A
	Coleoptera	Hybosoridae	*Phaeochroops rattus*	A
		Lampyridae	*unidentified sp.*	A
		Silphidae	*Chrysosilpha formosa*	A
		Staphylinidae	*Aleochara sp.*	A
	Dermaptera	Not identified	*unidentified sp.*	A

A = adult; L1 = 1st instar larvae; L2 = 2nd instar larvae; L3 = 3rd instar larvae; P = pupae; NE = newly emerged adult

4.1.3 STUDY 3

DECOMPOSITION PROCESS AND ARTHROPOD SUCCESSION ON MONKEY CARCASS IN OUTDOOR (NOCTURNAL)

Study 3 commenced on 1 August 2007 at 2200h and completed on 8 September 2007. The ambient temperature, relative humidity and daily rainfall amount during the Study 3 were shown in Figure 4.8 and Figure 4.9, respectively. The ambient temperature ranged from 20.4 to 31.9ºC (mean 25.38 ± 0.26ºC) and relative humidity ranged from 67 to 90% (mean 78.59 ± 0.75%). According to the Department of Meteorology Malaysia, 22 rainy days were recorded within 39 days of study period that accounted for daily rainfall amount ranging from 0.3 to 69.5 mm (mean 7.11 ± 2.36 mm).

The decomposition of carcass in Study 3 had undergone 5 stages namely, fresh (Day 1 – 3), bloating (Day 4 – 5), decay (Day 6), advanced decay (7 – 8) and remains (Day 9 – 39). The percentage of biomass remaining of monkey carcass is shown in Figure 4.10. The decomposition process caused decrease of body weight, leaving 35.71% of total body weight in the end of this study. The entomofauna obtained from monkey carcass in Study 3 is shown in Table 4.3.

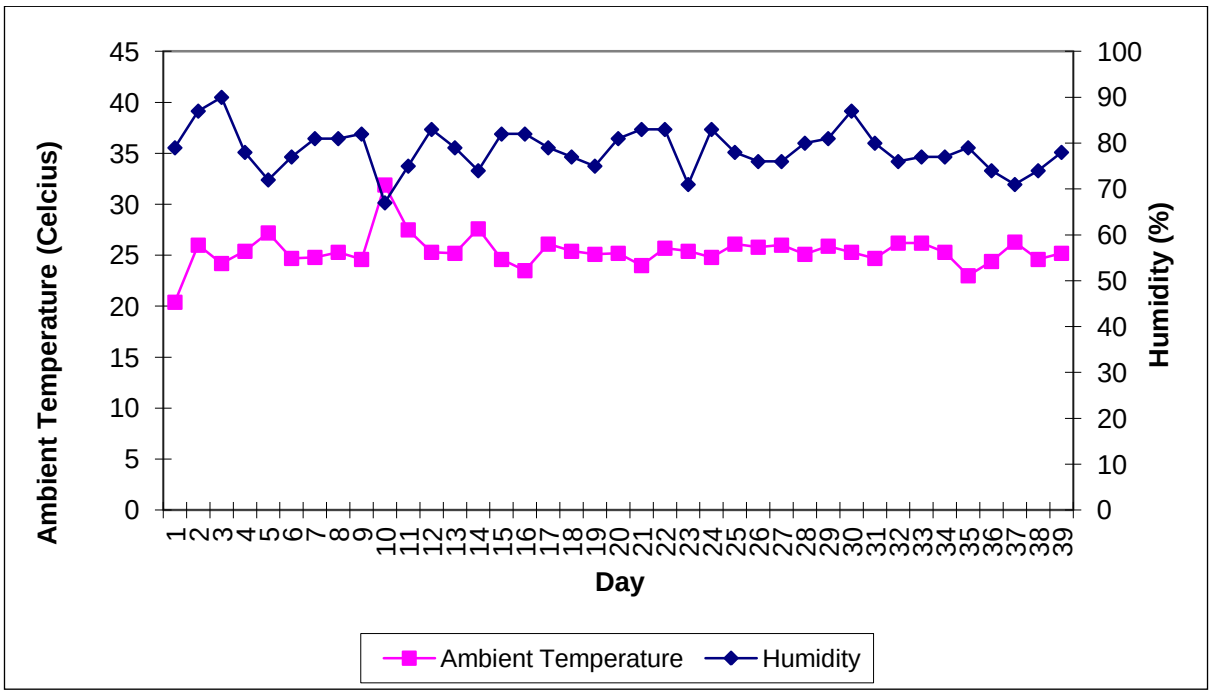

Figure 4.8. Ambient temperature and humidity of the study site during Study 3 conducted in Ulu Gombak, Selangor from 1 August 2007 – 8 September 2007 (39 Days).

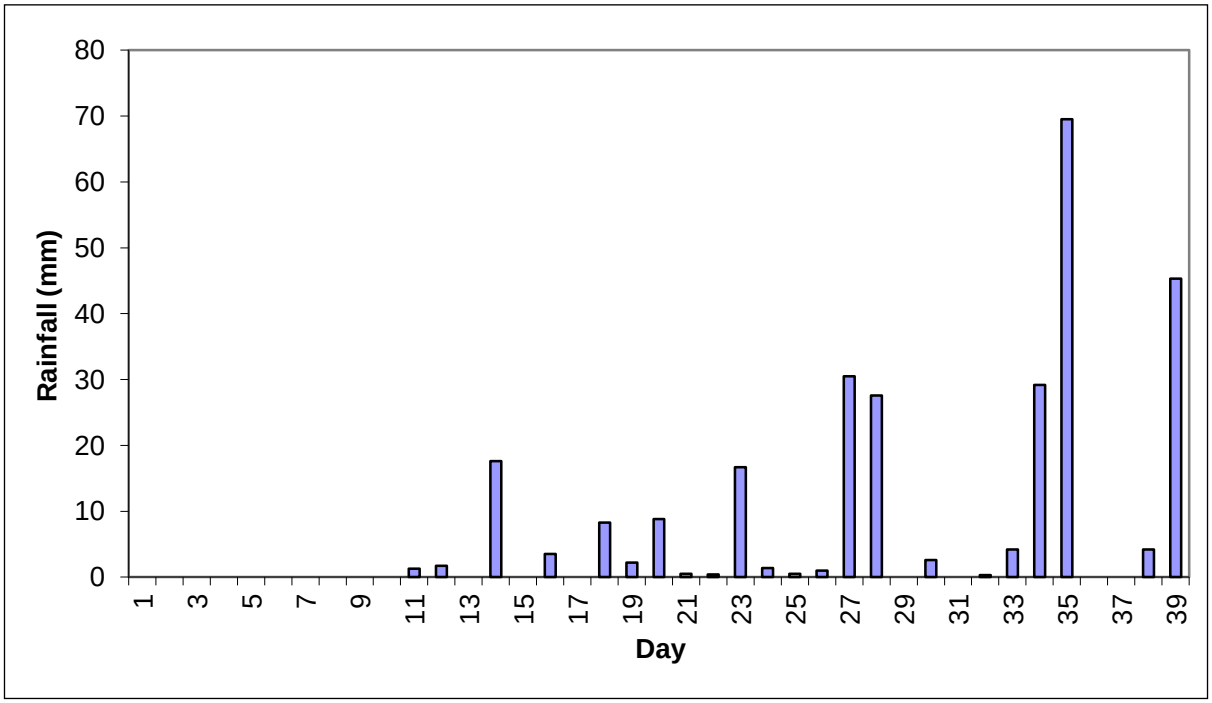

Figure 4.9. Daily rainfall amount in Ulu Gombak, Selangor during Study 3 conducted from 1 August 2007 – 8 September 2007 (39 Days). Data was provided by the Department of Meteorology Malaysia.

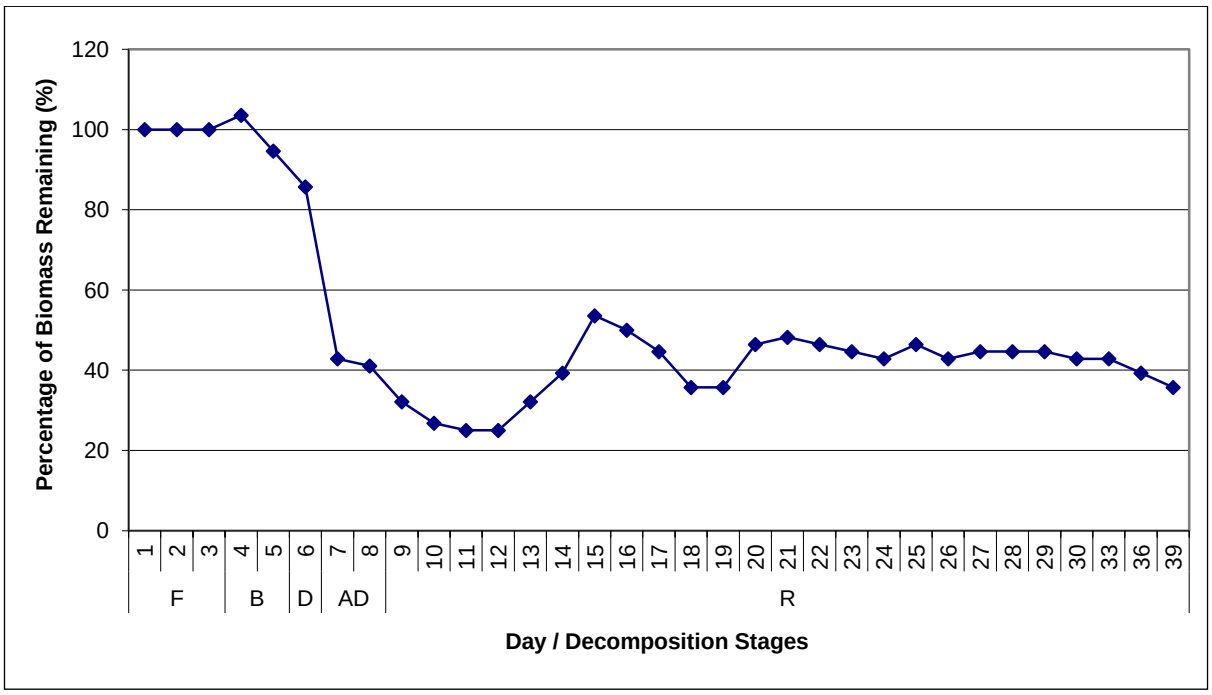

Figure 4.10. Percentage of biomass remaining of monkey carcass in Study 3 placed in lowland forested area located in Ulu Gombak, Selangor. The decomposition of carcass in Study 3 had undergone 5 stages namely, fresh (F), bloating (B), decay (D), advanced decay (AD) and remains (R).

Fresh stage (Day 1 – 3). The monkey was euthanised on Day 1 at 2200h. Within an hour, ants [*Pheidologeton diversus* (Hymenoptera: Formicidae: Mymicicnae) and *Odontoponera transversa* (Hymenoptera: Formicidae: Ponerinae)] were observed attracted to the bloodstain on the monkey carcass. Although the carcass was monitored hourly, however, no fly activity was observed throughout the night. On Day 2 at 0740h (sunrise time on 2 August 2007 was 0712h), females of *C. megacephala* were observed laying their eggs on the nose region of the carcass (1st oviposition). After this, many calliphorids were observed actively laying eggs on the nose, face, mouth, ears and neck regions of the carcass throughout the day before sunset. However, none of the eggs hatched on Day 2. On the other hand, many ants (*Pheidologeton diversus* and *Odontoponera transversa*) were observed predating and carrying away the fly eggs actively. No fly activities were observed after 1930h (sunset time on 2 August 2007 was 1927h). On Day 3, eggs on the ear region hatched at 0500h. Flies were observed to

begin visiting the carcass on 0745h (sunrise time on 3 August 2007 was 0712h). Masses of larvae were found in the mouth cavity and on the neck region at 1000h. The larvae were then identified as 1st- and 2nd-instar larvae of *H. violacea* and *C. megacephala*. No fly activities were observed after 1936h (sunset time on 3 August 2007 was 1927h). A beetle [*Phaeochroops rattus* (Coleoptera: Hybosoridae)] was found on the carcass at 2300h. Ants (*Pheidologeton diversus* and *Odontoponera transversa*) were observed predating and carrying away the fly eggs and larvae throughout the fresh stage.

Bloating stage (Day 4 – 5). The bloating stage was noticed on Day 4. Masses of larvae were found round the head region. The percentage of biomass remaining was slightly increased from Day 3 (100%) to Day 4 (103.57%). This may be due to the gases produced by the bacteria within the intestine or stomach trapped in the abdomen. The biomass begun to decrease once the abdomen of the carcass was exposed on Day 5. A total of four species of larvae were collected in bloating stage, namely *C. megacephala* (2nd-instar), *C. rufifacies* (2nd-instar), *C. pinguis* (3rd-instar) and *C. villeneuve* (3rd-instar). This stage lasted for 2 days.

Decay stage (Day 6). Strong decomposition odour was present. The number of calliphorids visiting the carcass decreased. The head region of carcass was almost completely consumed by the larvae. Only masses of 3rd-instar larvae of *C. pinguis* and *C. villeneuve* were found on the abdomen region of carcass. During this stage, beetles of family Silphidae (*Chrysosilpha formosa*) were found on the carcass. This stage lasted only for 1 day.

Advanced decay stage (Day 7 – 8). The advanced decay stage had minor odour and flesh was almost completely consumed by the larvae. Some of the skin layers were dehydrated. Third-instar larvae of *C. pinguis*, *C. villeneuve* and *C. chani* were obtained in this stage. Very few numbers of adult flies were observed visiting the carcass. Beetles (*Chrysosilpha formosa*) and ants (*Pheidologeton diversus* and *Odontoponera*

transversa) were found predating on the larvae. The advanced decay stage lasted for 2 days.

Remains stage (Day 9 – 39). The remains stage begun on Day 9 when majority of the bones and cartilages were observed. Less odour was noticed at this stage. Third-instar larvae of *Chrysomya nigripes*, *C. villeneuve* and *C. chani* were collected at the early remains stage (Day 9 – 13). The pupae of *C. villeneuve* and *C. chani* were found under the carcass on Day 10 and the newly emerged adult of *C. villeneuve* and *C. chani* were collected on Day 14. Third-instar larvae of *O. spinigera* were collected from Day 12 until Day 25; while larvae of *Hermetia illucens* (Diptera: Stratiomyiidae) were collected from Day 21 until the end of this study on Day 39. Beetles (*Chrysosilpha formosa*) and ants (*Pheidologeton diversus* and *Odontoponera transversa*) were found throughout the remains stage.

Table 4.3. Entomofauna attracted to various decomposition stages of monkey carcass (Study 3) placed in outdoor of lowland forested area located in Ulu Gombak, Selangor.

Decomposition stages (Duration, Day)	Order	Family	Species	Stage
Fresh (Day 1 – 3)	Diptera	Calliphoridae	*Hypopygiopsis violacea*	A, E, L1, L2
			Chrysomya megacephala	A, E, L1, L2
			Chrysomya pinguis	A
			Chrysomya villeneuve	A
	Hymenoptera	Formicidae	*Odontoponera transversa*	A
			Pheidologeton diversus	A
	Coleoptera	Hybosoridae	*Phaeochroops rattus*	A
Bloating (Day 4 – 5)	Diptera	Calliphoridae	*Chrysomya megacephala*	A, L2
			Chrysomya pinguis	A, L3
			Chrysomya rufifacies	L2
			Chrysomya villeneuve	A, L3
		Phoridae	*unidentified sp.*	A
	Hymenoptera	Formicidae	*Odontoponera transversa*	A
			Pheidologeton diversus	A
	Coleoptera	Hybosoridae	*Phaeochroops rattus*	A
Decay (Day 6)	Diptera	Calliphoridae	*Chrysomya megacephala*	A
			Chrysomya pinguis	L3
			Chrysomya villeneuve	A, L3
			Chrysomya chani	A
		Phoridae	*unidentified sp.*	A
	Hymenoptera	Formicidae	*Odontoponera transversa*	A
			Pheidologeton diversus	A
	Coleoptera	Silphidae	*Chrysosilpha formosa*	A
Advanced Decay (Day 7 – 8)	Diptera	Calliphoridae	*Chrysomya megacephala*	A
			Chrysomya pinguis	L3
			Chrysomya villeneuve	A, L3
			Chrysomya chani	A, L3
		Muscidae	*Ophyra spinigera*	A
		Phoridae	*unidentified sp.*	A
	Hymenoptera	Formicidae	*Odontoponera transversa*	A
			Pheidologeton diversus	A
	Coleoptera	Silphidae	*Chrysosilpha formosa*	A
Dry Remains (Day 9 – 39)	Diptera	Calliphoridae	*Chrysomya villeneuve*	L3, P, NE
			Chrysomya chani	L3, P, NE
			Chrysomya nigripes	L3
		Muscidae	*Ophyra spinigera*	L3
		Phoridae	*unidentified sp.*	A
		Stratiomyiidae	*Hermetia illucens*	L
	Hymenoptera	Formicidae	*Odontoponera transversa*	A
			Pheidologeton diversus	A
	Coleoptera	Silphidae	*Chrysosilpha formosa*	A

A = adult; L1 = 1st instar larvae; L2 = 2nd instar larvae; L3 = 3rd instar larvae; P = pupae; NE = newly emerged adult

4.1.4 STUDY 4

DECOMPOSITION PROCESS AND ARTHROPOD SUCCESSION ON MONKEY CARCASS IN INDOOR (NOCTURNAL)

Study 4 commenced simultaneously with Study 3 (serve as control) on 1st August 2007 at 2200h and completed on 25th October 2007. The ambient temperature, relative humidity and rainfall during the Study 4 are shown in Figure 4.11 and Figure 4.12, respectively. The ambient temperature ranged from 22.6 to 28.2ºC (mean 25.34 ± 0.21ºC) and relative humidity ranged from 57 to 94% (mean 70.83 ± 1.13%). The Department of Meteorology Malaysia recorded 51 rainy days within 86 days of study period that accounted daily rainfall amount ranged from 0.1 to 98.3 mm (mean 8.38 ± 1.77 mm).

The decomposition of carcass in Study 4 had undergone 5 stages namely, fresh (Day 1 – 3), bloating (Day 4 – 7), decay (Day 8 – 12), advanced decay (Day 13 – 27) and remains (Day 28 – 86). The percentage of biomass remaining of monkey carcass is shown in Figure 4.13. The decomposition process caused decrease of body weight, leaving only 32.14% of total body weight at the end of Study 4. The entomofauna obtained from monkey carcass in Study 4 is shown in Table 4.4.

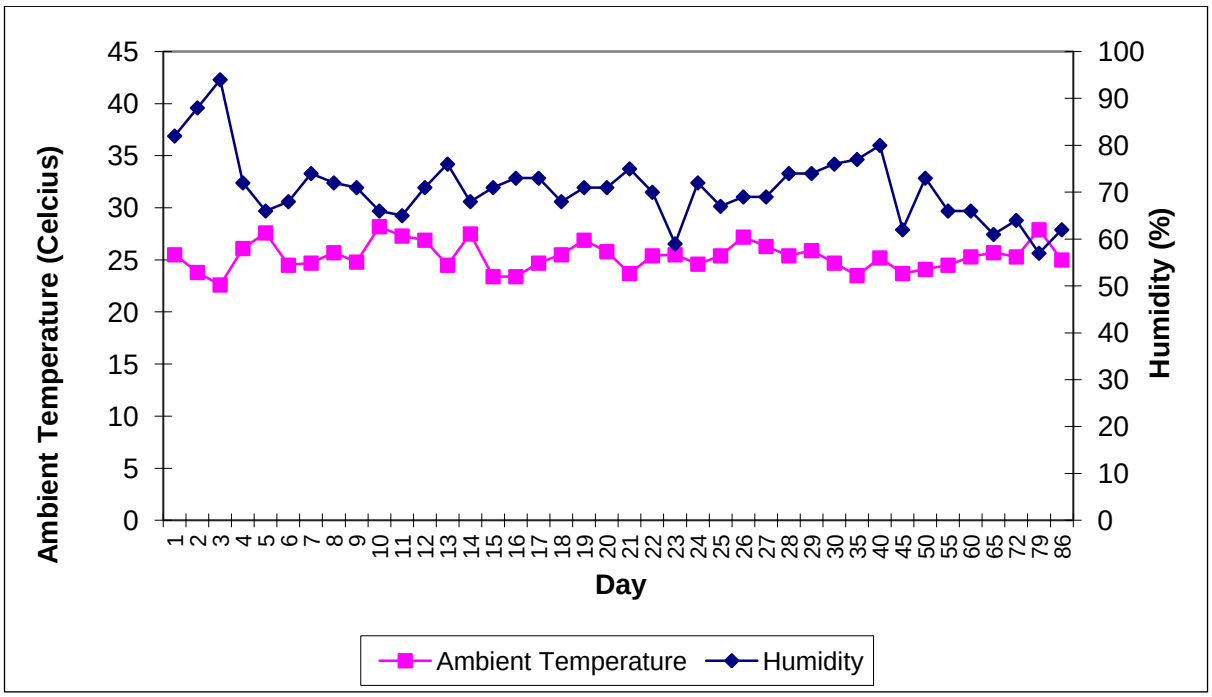

Figure 4.11. Indoor ambient temperature and humidity in the wooden hut during Study 4 conducted in Ulu Gombak, Selangor from 1 August 2007 – 25 October 2007 (86 Days).

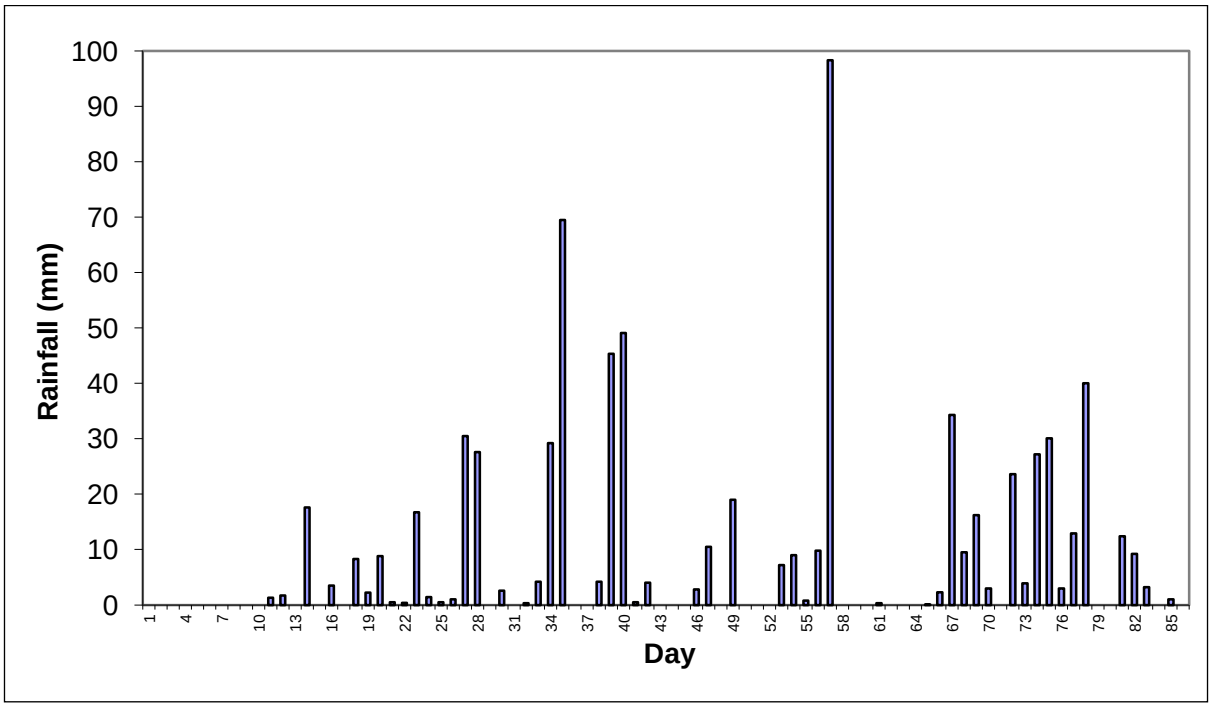

Figure 4.12. Daily rainfall amount in Ulu Gombak, Selangor during Study 4 conducted from 1 August 2007 – 25 October 2007 (86 Days). Data was provided by the Department of Meteorology Malaysia.

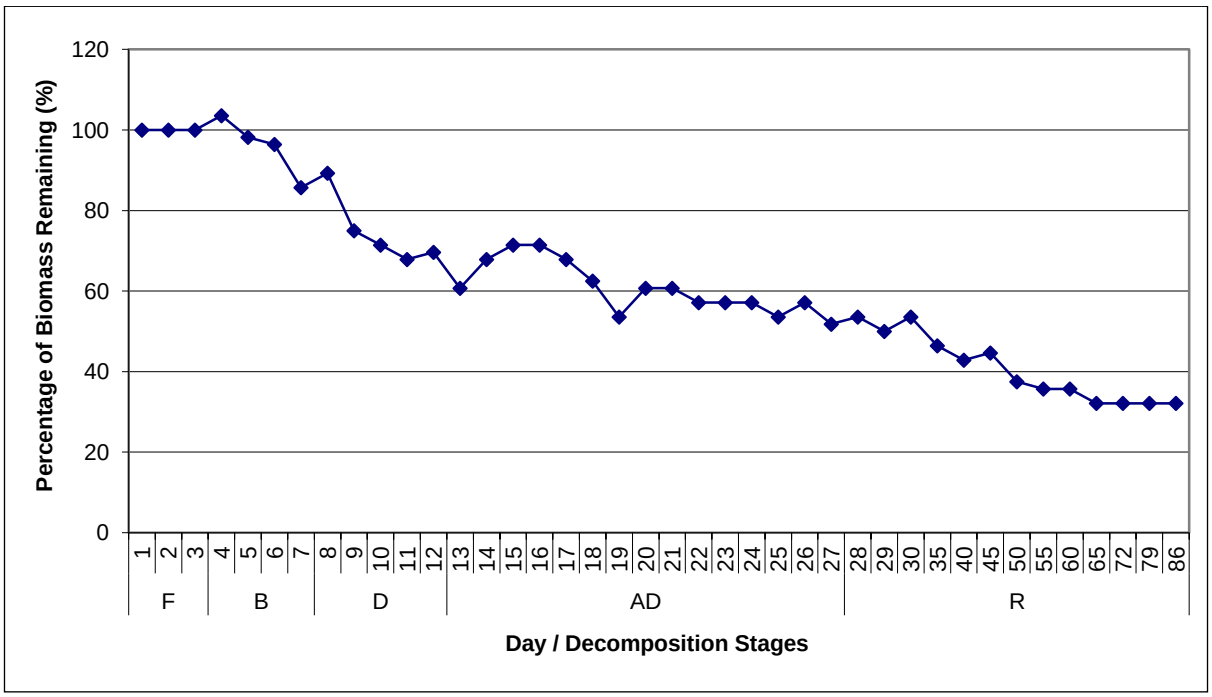

Figure 4.13. Percentage of biomass remaining of monkey carcass in Study 4 placed in lowland forested area located in Ulu Gombak, Selangor. The decomposition of carcass in Study 4 had undergone 5 stages namely, fresh (F), bloating (B), decay (D), advanced decay (AD) and remains (R).

Fresh stage (Day 1 – 3). On Day 1, the monkey was euthanised at 2200h. Ants (*Pheidologeton diversus* and *Odontoponera transversa*) were observed attracted to the bloodstain and the wound on the head of the carcass at 2300h, and this phenomenon continued throughout the fresh stage. No flies were observed visiting the carcass within the first three days.

Bloating stage (Day 4 – 7). On Day 4, a few adults of *C. megacephala* and *C. chani* were observed visiting the carcass. However, no eggs were observed on the carcass. The entire carcass was bloated and biomass increased about 3.57% due to the gases produced by the bacteria activities and trapped inside the abdomen. Strong odour was noticed due to the gases and liquids seen escaping from the wound caused by gun shot effect on the head region. On Day 5, second-instar larvae of *C. megacephala* were collected from the head region of carcass. The adult beetles, *Chrysosilpha formosa* (Coleoptera: Silphidae) and ants (*Pheidologeton diversus* and *Odontoponera*

transversa) were also observed visiting the carcass. On Day 6 and Day 7, larvae of *C. megacephala* (2nd- and 3rd-instar), *C. chani* (2nd-instar), *C. pinguis* (3rd-instar), *C. villeneuve* (3rd-instar) and *Hemipyrelia* sp. (3rd-instar) were obatiend. Many flies (more than 30 flies) were observed inside the hut when the decomposition odour was getting stronger on Day 6. The bloating stage lasted for 4 days.

Decay stage (Day 8 – 12). On Day 8, the carcass deflated and the stench from the carcass was strong. The number of flies (more than 50 flies) visited the carcass increased. Larvae masses containing *C. rufifacies*, *C. villeneuve*, *C. pinguis*, *C. chani* and *Hypopygiopsis* sp. were observed under the carcass. Some larvae were seen migrating from the carcass towards the corner of the hut for pupation. On Day 9, the monkey's hair detached from carcass, and pupae were collected from the corner inside the hut and under the carcass. On Day 10, third-instar larvae of *O. spinigera* were present. Three species of adult coleopteran [*Platydracus* sp. (Staphylinidae), *Chrysosilpha formosa* (Silphidae) and *Phaeochroops peninsularis* (Hybosoridae)] and two species of ants (*Pheidologeton diversus* and *Odontoponera transversa*) were obtained throughout the decay stage. This stage lasted for 5 days.

Advanced decay stage (13 – 27). In the advanced decay stage, most of the flesh had been consumed and the odour had faded. Many larvae and pupae were observed under the carcass, and pupae were also found around the corner of the hut. Ants were seen predating on the larvae and pupae. On Day 15, newly emerged adults of *C. chani*, *C. megacephala* and *C. villeneuve* were collected. On the other hand, the carcass begun to mummify when the derma on fore and hind limbs began to darken and dry out. At this stage, the carcass was dominanted by *O. spinigera* larvae, and beetle larvae, *Chrysosilpha formosa* (Silphidae). The decomposition process was slow, and biomass was reduced to 51.79% by weight at the end of advanced decay stage. This stage lasted for 15 days.

Remains stage (Day 28 – 86). Mummification was observed in this stage. No fly was observed inside the hut. On Day 29, *Hermetia illucens* larvae emerged from the carcass. At the beginning of remains stage, the carcass was dominated by larvae of *O. spinigera* and *Hermetia illucens*; however, only *Hermetia illucens* larvae were obtained from the carcass from Day 51 onwards until the end of this study which was on Day 86. Beetles belonging to the family Silphidae (*Chrysosilpha formosa*) and Tenebrionidae (unidentified species) were collected in this stage. Larvae of *Chrysosilpha formosa* were found to colonise the mummified carcass. The remains stage lasted for 59 days.

Table 4.4. Entomofauna attracted to various decomposition stages of monkey carcass (Study 4) placed in indoor of lowland forested area located in Ulu Gombak, Selangor.

Decomposition stages (Duration, Day)	Order	Family	Species	Stage
Fresh (Day 1 – 3)	Hymenoptera	Formicidae	*Odontoponera transversa*	A
			Pheidologeton diversus	A
Bloating (Day 4 – 7)	Diptera	Calliphoridae	*Chrysomya chani*	A, L2
			Chrysomya megacephala	A, L2, L3
			Chrysomya pinguis	L3
			Chrysomya villeneuve	A, L3
			Hemipyrelia sp.	L3
		Muscidae	*Ophyra spinigera*	A
		Phoridae	*unidentified sp.*	A
	Hymenoptera	Formicidae	*Odontoponera transversa*	A
			Pheidologeton diversus	A
	Coleoptera	Silphidae	*Chrysosilpha formosa*	A
Decay (Day 8 – 12)	Diptera	Calliphoridae	*Chrysomya chani*	A, L3, P
			Chrysomya megacephala	A, L3, P
			Chrysomya pinguis	L3
			Chrysomya villeneuve	A, L3, P
			Hypopygiopsis violacea	L3
		Muscidae	*Ophyra spinigera*	A, L3
		Phoridae	*unidentified sp.*	A
	Hymenoptera	Formicidae	*Odontoponera transversa*	A
			Pheidologeton diversus	A
	Coleoptera	Hybosoridae	*Phaeochroops peninsularis*	A
		Silphidae	*Chrysosilpha formosa*	A
		Staphylinidae	*Platydracus sp.*	A
Advanced Decay (Day 13 – 27)	Diptera	Calliphoridae	*Chrysomya chani*	A, L3, P, NE
			Chrysomya megacephala	A, L3, P, NE
			Chrysomya pinguis	L3
			Chrysomya villeneuve	A, P, NE
			Hypopygiopsis violacea	L3
		Muscidae	*Ophyra spinigera*	A, L3, P
		Phoridae	*unidentified sp.*	A
	Hymenoptera	Formicidae	*Odontoponera transversa*	A
	Coleoptera	Lycidae	*unidentified sp.*	A
		Silphidae	*Chrysosilpha formosa*	A, L
Dry Remains (Day 28 – 86)	Diptera	Calliphoridae	*Chrysomya chani*	L3, P, NE
		Muscidae	*Ophyra spinigera*	L3, P, NE
		Stratiomyiidae	*Hermetia illucens*	L
	Coleoptera	Silphidae	*Chrysosilpha formosa*	A, L
		Tenebrionidae	*unidentified sp.*	A

A = adult; L1 = 1st instar larvae; L2 = 2nd instar larvae; L3 = 3rd instar larvae; P = pupae; NE = newly emerged adult

4.2 TANJONG SEPAT (COASTAL AREA)

A total of four studies were conducted in coastal area located in Tanjong Sepat, Selangor. Study 5 (Outdoor – Replicate 1) was designed to study the decomposition process and arthropod succession on monkey carcass placed in outdoor (control); and for comparison purpose, Study 6 (Indoor – Replicate 1) was designed to study the decomposition process and arthropod succession on the monkey carcass placed in indoor. Both studies commenced on daytime from 6 May 2008, 1100h and continued until 23 May 2008.

The study was repeated from 22 July 2008, 1100h until 5 August 2008, known as Study 7 (Outdoor – Replicate 2) and Study 8 (Indoor – Replicate 2), conducted in outdoor and indoor, respectively. Because flies were inactive during nighttime, thus all studies in coastal area were conducted during the diurnal period only.

4.2.1 STUDY 5 AND STUDY 7

DECOMPOSITION PROCESS AND ARTHROPOD SUCCESSION ON MONKEY CARCASS IN OUTDOOR (REPLICATE 1 AND 2)

Both Study 5 and Study 7 were conducted on daytime, and no significant difference was observed on ambient temperature, relative humidity and daily rainfall amount (Table 4.5) during the course of study; thus, the observation and results of both studies were combined and explained in this section.

Table 4.5. Comparison on mean outdoor ambient temperature, relative humidity and daily rainfall amount between forensic entomological studies (Study 5 and Study 7) conducted in coastal area of Tanjong Sepat, Selangor.

Meteorology	Study 5 (Replicate 1) 6 – 14 May 2008	Study 7 (Replicate 2) 22 – 30 July 2008	t-test
Temperature, ºC	32.04 ± 0.86	30.05 ± 0.56	P = 0.070, t = 1.939, df = 16
Humidity, %	58.33 ± 3.35	64.89 ± 2.21	P = 0.122, t = -1.635, df = 16
Rainfall, mm	2.89 ± 1.45	5.03 ± 1.26	P = 0.282, t = -1.114, df = 16

$p > 0.05$: not significantly difference $p \leq 0.05$: significantly difference

Study 5 was carried out on 6 May 2008 at 1100h and completed on 14 May 2008 (within a time frame of 9 days). The ambient temperature, relative humidity and daily rainfall amount during Study 5 are shown in Figure 4.14 and Figure 4.15, respectively. The ambient temperature ranged from 26.8 to 36.3ºC (mean 32.04 ± 0.86ºC) and relative humidity ranged from 41 to 76% (mean 58.33 ± 3.35%). According to the record of Department of Meteorology Malaysia, 7 rainy days was recorded within 9 days of study period that accounted daily rainfall amount ranging from 0.4 to 12.1mm (mean 2.89 ± 1.45mm).

The decomposition of carcass in Study 5 had undergone 5 stages, namely fresh (Day 1 – 2), bloating (Day 3), decay (Day 4) advanced decay (Day 5) and remains (Day 6 – 9). The percentage of biomass remaining of monkey carcass in each decomposition stage is shown in Figure 4.16. The decomposition process caused decrease of body weight, leaving only 17.24% of total body weight in the end of this study. The entomofauna obtained from monkey carcass in Study 5 is shown in Table 4.6.

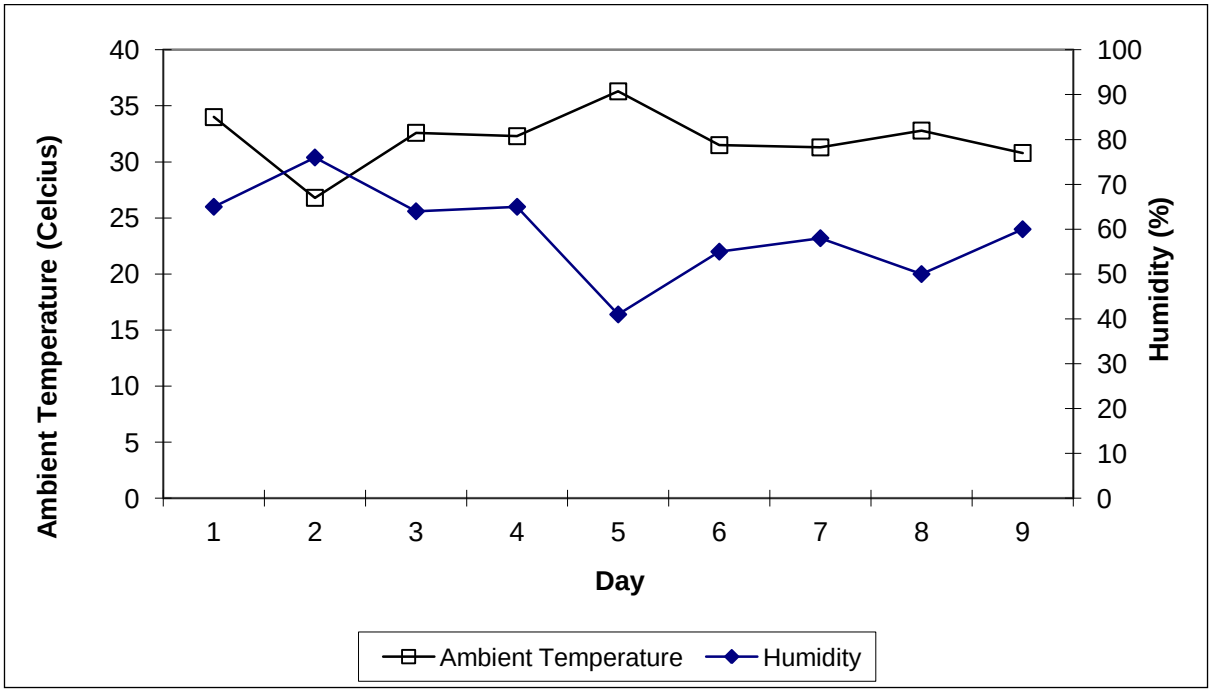

Table 4.14. Ambient temperature and humidity of the study site during Study 5 conducted in Tanjong Sepat, Selangor from 6 – 14 May 2008 (9 Days).

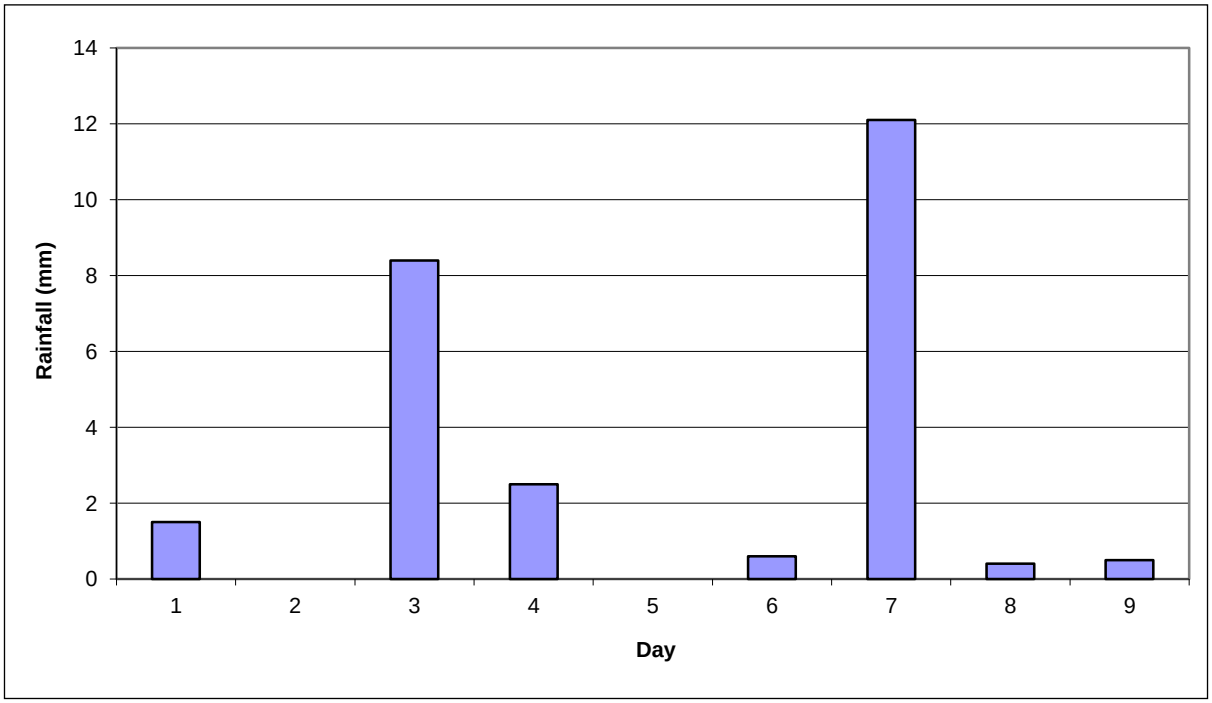

Figure 4.15. Daily rainfall amount in Tanjong Sepat, Selangor during Study 5 conducted from 6 – 14 May 2008 (9 Days). Data was provided by the Department of Meteorology Malaysia.

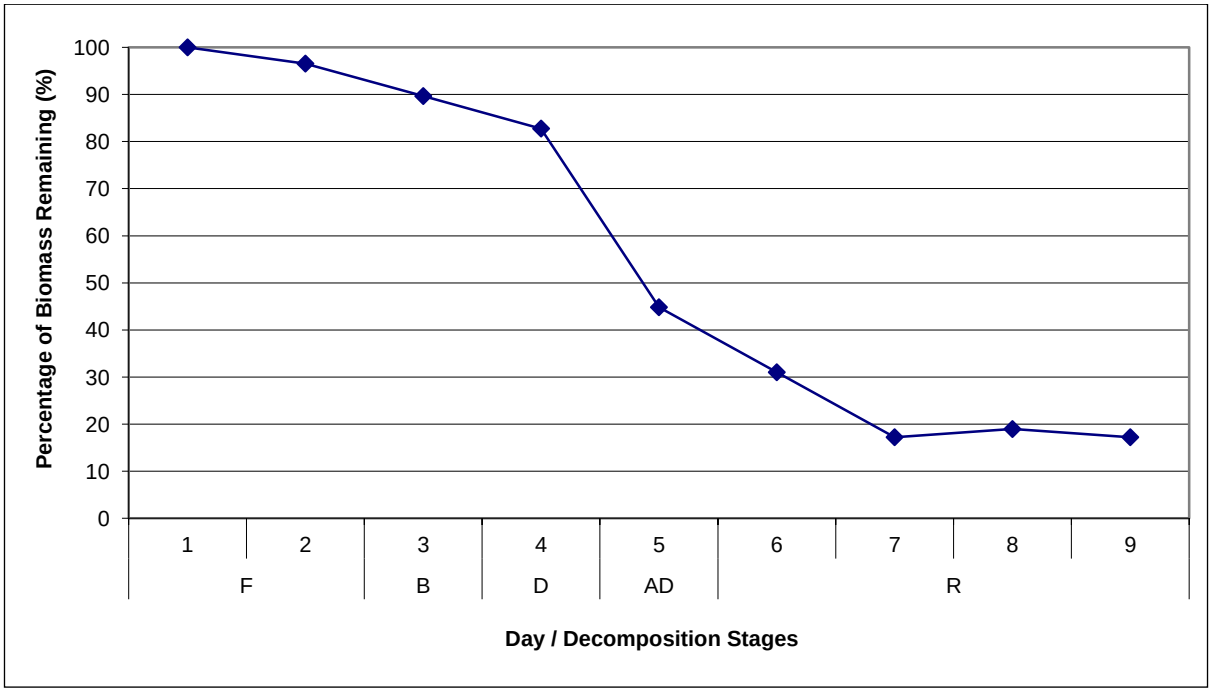

Figure 4.16. Percentage of biomass remaining of monkey carcass in Study 5 placed in coastal area located in Tanjong Sepat, Selangor. The decomposition of carcass in Study 5 had undergone 5 stages, namely fresh (F), bloating (B), decay (D), advanced decay (AD) and remains (R).

Study 7 was carried out on 22 July 2008 at 1100h and completed on 30 July 2008 (time frame of 9 days). The ambient temperature, relative humidity and daily rainfall amount during the Study 7 are shown in Figure 4.17 and Figure 4.18, respectively. The ambient temperature ranged from 26.0 to 31.5ºC (mean 30.05 ± 0.56ºC) and relative humidity ranged from 53 to 74% (mean 64.89 ± 2.21%). According to the Department of Meteorology Malaysia, 7 rainy days were recorded within 9 days of study period that accounted daily rainfall ranged from 1.9 to 10.5mm (mean 5.03 ± 1.26mm).

The decomposition of carcass in Study 7 had undergone 5 stages, namely, fresh (Day 1), bloating (Day 2), decay (Day 3), advanced decay (Day 4) and remains (Day 5 – 9). The percentages of biomass remaining of monkey carcass are shown in Figure 4.19. The decomposition process caused decrease of body weight, leaving 12.90% of total body weight in the end of this study. The entomofauna obtained from monkey carcass in Study 7 is shown in Table 4.7.

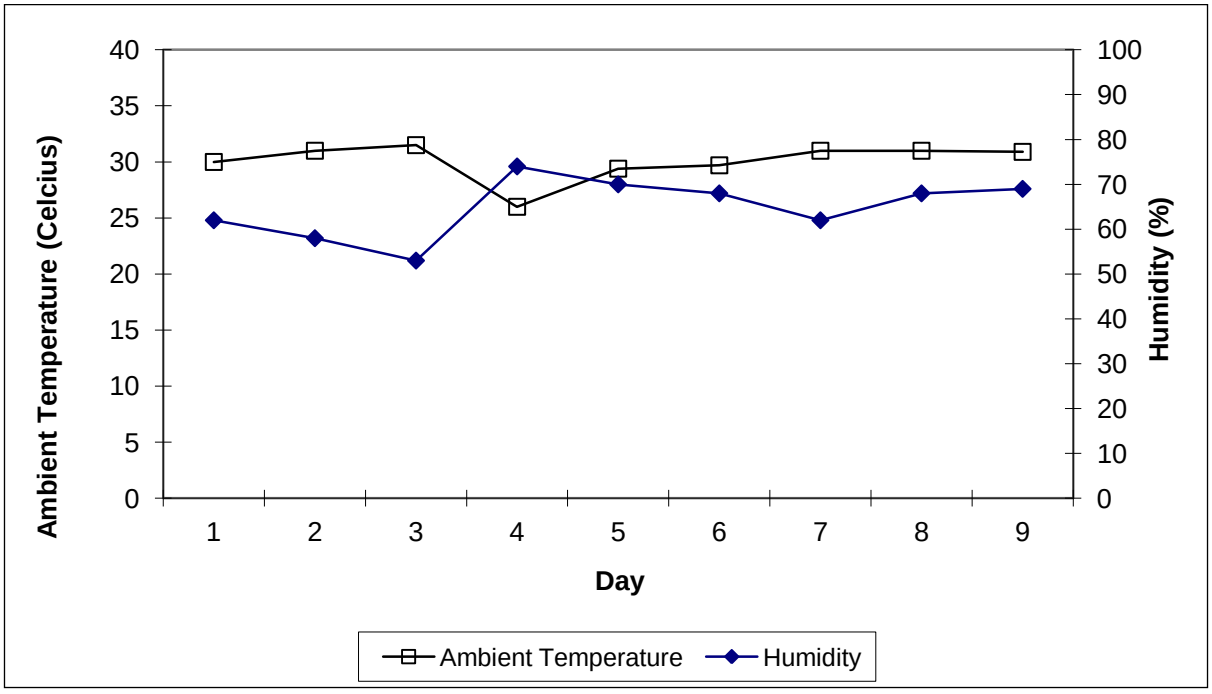

Figure 4.17. Ambient temperature and humidity of the study site during Study 7 conducted in Tanjong Sepat, Selangor from 22 – 30 July 2008 (9 Days).

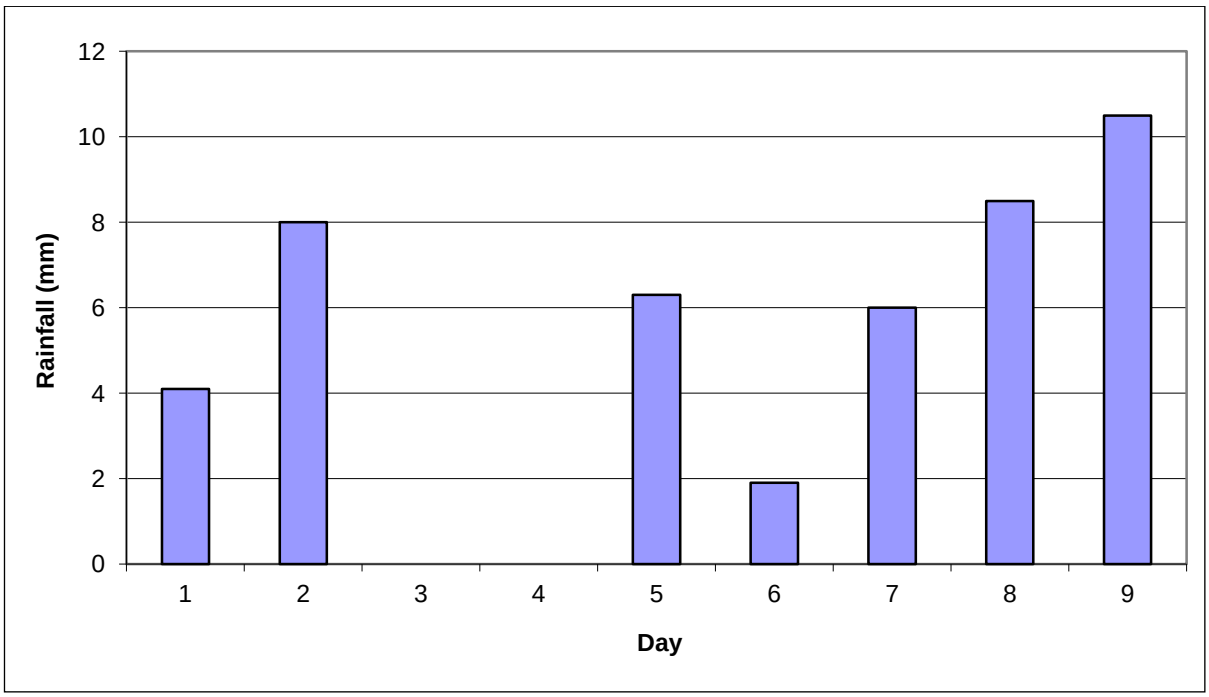

Figure 4.18. Daily rainfall amount in Tanjong Sepat, Selangor during Study 7 conducted from 22 – 30 July 2008 (9 Days). Data was provided by the Department of Meteorology Malaysia.

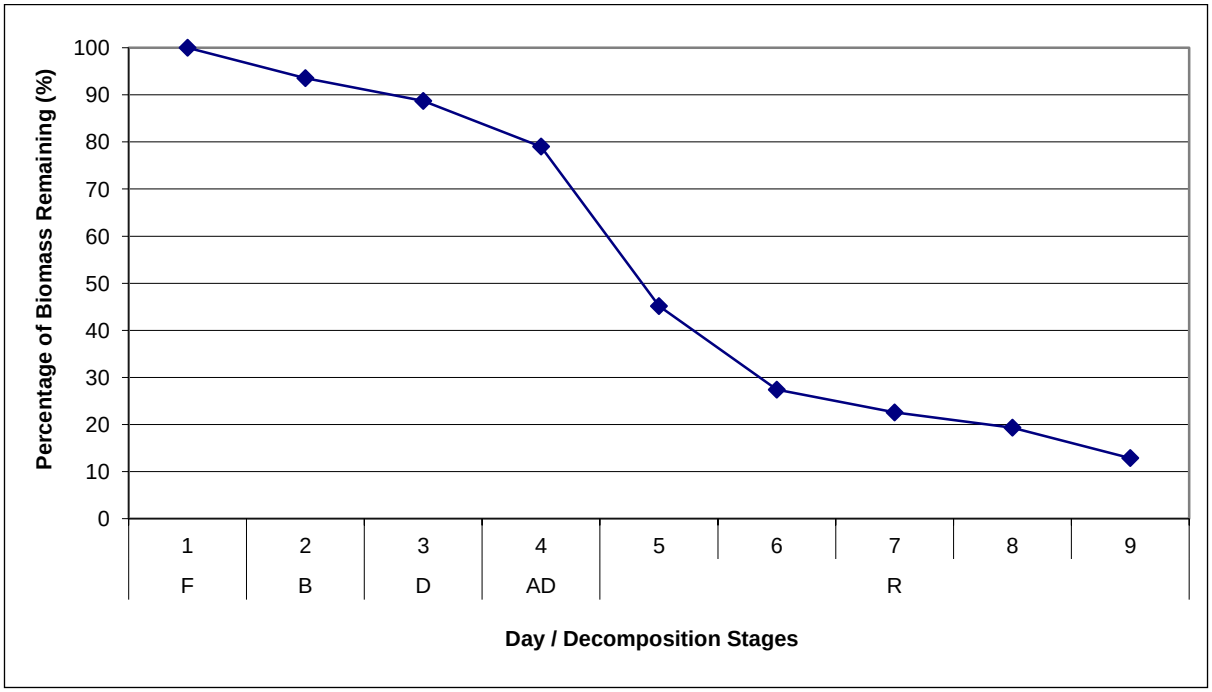

Figure 4.19. Percentage of biomass remaining of monkey carcass in Study 7 placed in coastal area located in Tanjong Sepat, Selangor. The decomposition of carcass in Study 7 had undergone 5 stages, namely fresh (F), bloating (B), decay (D), advanced decay (AD) and remains (R).

Fresh stage (Study 5: Day 1 – 2 / Study 7: Day 1). The monkey carcasses for both studies were placed on the ground of sandy beach at 1100h. Within a minute, the muscid fly, *Musca domestica* was the first visitor attracted to the monkey carcass, followed by *Sarcophaga* spp., *Chrysomya megacephala* and *Hemipyrelia* spp. Flies laid eggs on the mouth, nose and face regions of monkey carcasses at 2 hours (1300h) and 4 hours 50 minutes (1550h) after the carcasses placed on the ground in Study 5 and Study 7, respectively.

For Study 5, the ants (Hymenoptera: Formicidae), namely *Cardiocondyla* spp., *Oecophylla smaragdina* and *Pheidologeton* spp., were present around the carcass at 1400h. Flies belonging to the family Phoridae were observed after 5 hours (1600h), and no fly activity was observed after 1700h due to rain. On Day 2, about 20 flies consisting of *Chrysomya rufifacies*, *Chrysomya megacephala*, *Hemipyrelia* spp., *Phumosia* spp., *Musca domestica*, *Sarcophaga* spp., Phorid flies and *Scholastes* sp. were seen visiting the carcass. First- and second-instar larvae of *Chrysomya megacephala* and *Hemipyrelia* sp. were collected from the mouth region. A frog (Amphibia: Anura) was seen resting beside the carcass, presumably a predator for the adult flies attracted to the carcass. Ants were also seen predating on the fly eggs and larvae.

For Study 7, the ants were seen predating the fly eggs immediately after they were laid on the mouth and face regions at 1600h. A hermit crab, *Coenobita* spp. (Crustacea: Decapoda: Coenobitidae) was seen crawling near the carcass.

The fresh stage in Study 5 and Study 7 lasted for 2 and 1 day, respectively.

Bloating stage (Study 5: Day 3 / Study 7: Day 2). Bloating stage was noticed once the carcass's abdomen began to bloat. In Study 5, larvae of *Hemipyrelia* sp. (3rd-instar), *C. megacephala* (2nd-instar) and *C. rufifacies* (1st-instar) were collected from the head region; while in Study 7, only larvae of *Hemipyrelia* spp. (2nd-instar) were collected from mouth region. Thirty to fourty adults of *Hemipyrelia* spp., *C.*

megacephala, *C. rufifacies*, *M. domestica* and *Sarcophaga* spp. were observed around both carcasses. *Ophyra spinigera* was seen to visit the carcass in Study 7 from bloating stage until the end of study. Bloating stage in both studies only lasted for a day.

Decay stage (Study 5: Day 4 / Study 7: Day 3). The decay stage begun when the stomach contents were exposed. Approximately 100 flies were seen visiting the carcasses. In Study 5, maggot masses of *C. megacephala* (3rd-instar) and *C. rufifacies* (2nd-instar) were found on mouth and around anus regions. Larvae of *C. rufifacies* were dominant in the maggot masses. *Ophyra spinigera* adults were attracted to the carcass from this stage onwards. Neriid (Diptera: Neriidae) flies were observed licking fluid on the carcass. In Study 7, only maggot mass of *Hemipyrelia* spp. (3rd-instar) was found colonising at mouth region actively. The jaws of the carcass were exposed. Egg masses of *C. megacephala* and *C. rufifacies* were seen on the armpit and chest regions. Ants were seen predating on the fly's eggs and larvae actively. The decay stage in both studies lasted for a day.

Advanced decay stage (Study 5: Day 5 / Study 7: Day 4). In this stage, the skull and ribs were exposed. Most of the flesh, internal organs and tissues were consumed by larvae. Third- and second-instar larvae of *C. megacephala* and *C. rufifacies* were found on the carcass in Study 5 and Study 7. Only adults (about 40 flies) of *C. megacephala*, *C. rufifacies* and *Ophyra spinigera* were obtained from this stage. The skin layer of carcass begun to dry out, due to hot weather and wind in the study site. This stage lasted for a day. Four hermit crabs, *Coenobita* sp. (Crustacea: Decapoda: Coenobitidae) were seen on the rotting regions of monkey carcass in Study 7. The advanced decay stage in both studies lasted for a day.

Remains stage (Study 5: Day 6 – 9 / Study 7: Day 5 – 9). Both carcasses in Study 5 and Study 7 entered remains stage on Day 6 and Day 5, respectively. In this stage, the carcasses were dehydrated and mummified. Less odour was noticed.

In Study 5, 3rd- and 2nd-instar larvae of *C. rufifacies* and *O. spinigera* were collected on Day 6, respectively. Pupae of *C. rufifacies* were found on and under the carcass. Very few adult flies (less than 10) visited the carcass. On Day 7 onwards, no adult flies were observed. Third-instar larvae of *O. spinigera* were dominant and hiding under the carcass until end of the study. Newly emerged adult flies of *C. rufifacies* were seen resting on the carcass on Day 10. Ants were seen to predate on the newly emerged adult flies.

In Study 7, 3rd-instar larvae of *C. rufifacies* and *O. spinigera* were collected on Day 5. *Chrysomya rufifacies* larvae burrowed into the sand (3 – 5 cm depth) for pupation. Very few adult flies (less than 10) visited the carcass. On Day 6, *C. rufifacies* pupae were collected from the sand under the carcass. Only muscids (*Musca domestica* and *Ophyra spinigera*) were seen to visit the carcass from Day 6 onwards. On Day 7, *Ophyra spinigera* (2nd- and 3rd-instar) larvae were collected until the end of this study. Newly emerged adults of *C. megacephala* and *C. rufifacies* were collected on Day 9 and Day 10, respectively. Ants were seen to predate on the newly emerged adult flies.

No larvae were observed after Day 9 in both studies. Both studies were terminated on Day 10 when no larvae were found on the carcass.

Table 4.6. Entomofauna attracted to various decomposition stages of monkey carcass (Study 5) placed in outdoor of coastal area located in Tanjong Sepat, Selangor.

Decomposition stages (Duration, Day)	Order	Family	Species	Stage
Fresh (Day 1 – 2)	Diptera	Calliphoridae	*Chrysomya megacephala*	A, E, L1, L2
			Chrysomya rufifacies	A, E
			Hemipyrelia sp.	A, E, L1, L2
			Phumosia sp.	A
		Muscidae	*Musca domestica*	A
		Phoridae	*unidentified sp.*	A
		Platystomatidae	*Scholastes sp.*	A
		Sarcophagidae	*unidentified sp.*	A
	Hymenoptera	Formicidae	*Cardiocondyla sp.*	A
			Oecophylla smaragdina	A
			Pheidologeton sp.	A
Bloating (Day 3)	Diptera	Calliphoridae	*Chrysomya megacephala*	A, L2
			Chrysomya rufifacies	A, L1
			Hemipyrelia sp.	A, L3
		Muscidae	*Musca domestica*	A
		Sarcophagidae	*unidentified sp.*	A
	Hymenoptera	Formicidae	*Cardiocondyla sp.*	A
			Oecophylla smaragdina	A
			Pheidologeton sp.	A
Decay (Day 4)	Diptera	Calliphoridae	*Chrysomya megacephala*	A, L3
			Chrysomya rufifacies	A, L2
		Muscidae	*Musca domestica*	A
			Ophyra spinigera	A
		Neriidae	*unidentified sp.*	A
		Sarcophagidae	*unidentified sp.*	A
	Hymenoptera	Formicidae	*Cardiocondyla sp.*	A
			Oecophylla smaragdina	A
			Pheidologeton sp.	A
Advanced Decay (Day 5)	Diptera	Calliphoridae	*Chrysomya megacephala*	A, L3
			Chrysomya rufifacies	A, L3
		Muscidae	*Ophyra spinigera*	A
	Hymenoptera	Formicidae	*Cardiocondyla sp.*	A
			Oecophylla smaragdina	A
			Pheidologeton sp.	A
Dry Remains (Day 6 – 10)	Diptera	Calliphoridae	*Chrysomya megacephala*	A
			Chrysomya rufifacies	A, L3, P, NE
		Muscidae	*Ophyra spinigera*	A, L2, L3
		Neriidae	*unidentified sp.*	A
		Sarcophagidae	*unidentified sp.*	A
	Hymenoptera	Formicidae	*Cardiocondyla sp.*	A
			Oecophylla smaragdina	A
			Pheidologeton sp.	A

A = adult; L1 = 1st instar larvae; L2 = 2nd instar larvae; L3 = 3rd instar larvae; P = pupae; NE = newly emerged adult

Table 4.7. Entomofauna attracted to various decomposition stages of monkey carcass (Study 7) placed in outdoor of coastal area located in Tanjong Sepat, Selangor.

Decomposition stages (Duration, Day)	Order	Family	Species	Stage
Fresh (Day 1)	Diptera	Calliphoridae	*Chrysomya megacephala*	A
			Chrysomya rufifacies	A
			Hemipyrelia sp.	A, E
		Muscidae	*Musca domestica*	A
		Phoridae	*unidentified sp.*	A
		Sarcophagidae	*unidentified sp.*	A
	Hymenoptera	Formicidae	*Cardiocondyla sp.*	A
			Oecophylla smaragdina	A
			Pheidologeton sp.	A
Bloating (Day 2)	Diptera	Calliphoridae	*Chrysomya megacephala*	A
			Chrysomya rufifacies	A
			Hemipyrelia sp.	A, L2
		Muscidae	*Musca domestica*	A
			Ophyra spinigera	A
		Phoridae	*unidentified sp.*	A
		Sarcophagidae	*unidentified sp.*	A
	Hymenoptera	Formicidae	*Cardiocondyla sp.*	A
			Oecophylla smaragdina	A
			Pheidologeton sp.	A
Decay (Day 3)	Diptera	Calliphoridae	*Chrysomya megacephala*	A, E
			Chrysomya rufifacies	A, E
			Hemipyrelia sp.	A, L3
		Muscidae	*Musca domestica*	A
			Ophyra spinigera	A
		Sarcophagidae	*unidentified sp.*	A
	Hymenoptera	Formicidae	*Cardiocondyla sp.*	A
			Oecophylla smaragdina	A
			Pheidologeton sp.	A
Advanced Decay (Day 4)	Diptera	Calliphoridae	*Chrysomya megacephala*	A, L2
			Chrysomya rufifacies	A, L2
		Muscidae	*Ophyra spinigera*	A
	Hymenoptera	Formicidae	*Cardiocondyla sp.*	A
			Oecophylla smaragdina	A
			Pheidologeton sp.	A
Dry Remains (Day 5 – 9)	Diptera	Calliphoridae	*Chrysomya megacephala*	A, L3, P, NE
			Chrysomya rufifacies	A, L3, P, NE
		Muscidae	*Ophyra spinigera*	A, L2, L3
		Neriidae	*unidentified sp.*	A
		Sarcophagidae	*unidentified sp.*	A
	Hymenoptera	Formicidae	*Cardiocondyla sp.*	A
			Oecophylla smaragdina	A
			Pheidologeton sp.	A

A = adult; L1 = 1st instar larvae; L2 = 2nd instar larvae; L3 = 3rd instar larvae; P = pupae; NE = newly emerged adult

4.2.2 STUDY 6 AND STUDY 8

DECOMPOSITION PROCESS AND ARTHROPOD SUCCESSION ON MONKEY CARCASS IN INDOOR (REPLICATE 1 AND REPLICATE 2)

Both Study 6 and 8 commenced on daytime because no significant difference was observed on ambient temperature, relative humidity and daily rainfall (Table 4.8) in these studies and the results of both studies were combined and explained in this section.

Table 4.8. Comparison on mean indoor ambient temperature, relative humidity and daily rainfall amount between forensic entomological studies (Study 6 and Study 8) conducted in a wooden hut (indoor) located in coastal area of Tanjong Sepat, Selangor.

Meteorology	Study 6 (Replicate 1) 6 – 23 May 2008	Study 8 (Replicate 2) 22 July – 5 August 2008	t-test
Temperature, ºC	32.82 ± 0.65	31.05 ± 0.55	P = 0.051, t = 2.030, df = 31
Humidity, %	56.56 ± 2.16	62.07 ± 2.45	P = 0.101, t = -1.692, df = 31
Rainfall, mm	1.97 ± 0.78	4.80 ± 1.26	P = 0.057, t = -1.977, df = 31

$p > 0.05$: not significantly difference $p \leq 0.05$: significantly difference

Study 6 commenced simultaneously with Study 5 (which served as a control) on 6 May 2008 at 1130h and completed on 23 May 2008 (within a time frame of 18 days). The indoor ambient temperature, relative humidity and daily rainfall amount during Study 6 are shown in Figure 4.20 and Figure 4.21, respectively. The indoor ambient temperature ranged from 26.6 to 37.7ºC (mean 32.82 ± 0.65ºC) and relative humidity ranged from 35 to 74% (mean 56.56 ± 2.16%). According to the Department of Meteorology Malaysia, 11 rainy days was recorded within 18 days of study period that accounted daily rainfall amount ranged from 0.4 to 12.1mm (mean 1.97 ± 0.78mm).

The decomposition of the carcass in Study 6 had undergone 5 stages, namely, fresh (Day 1 – 2), bloating (Day 3), decay (Day 4), advanced decay (Day 5) and remains (Day 6 – 18). The percentage of biomass remaining of the monkey carcass placed indoor was shown in Figure 4.22. The decomposition process caused decrease of body

weight, leaving only 13.33% of total body weight at the end of this study. The entomofauna obtained from monkey carcass in Study 6 is shown in Table 4.9.

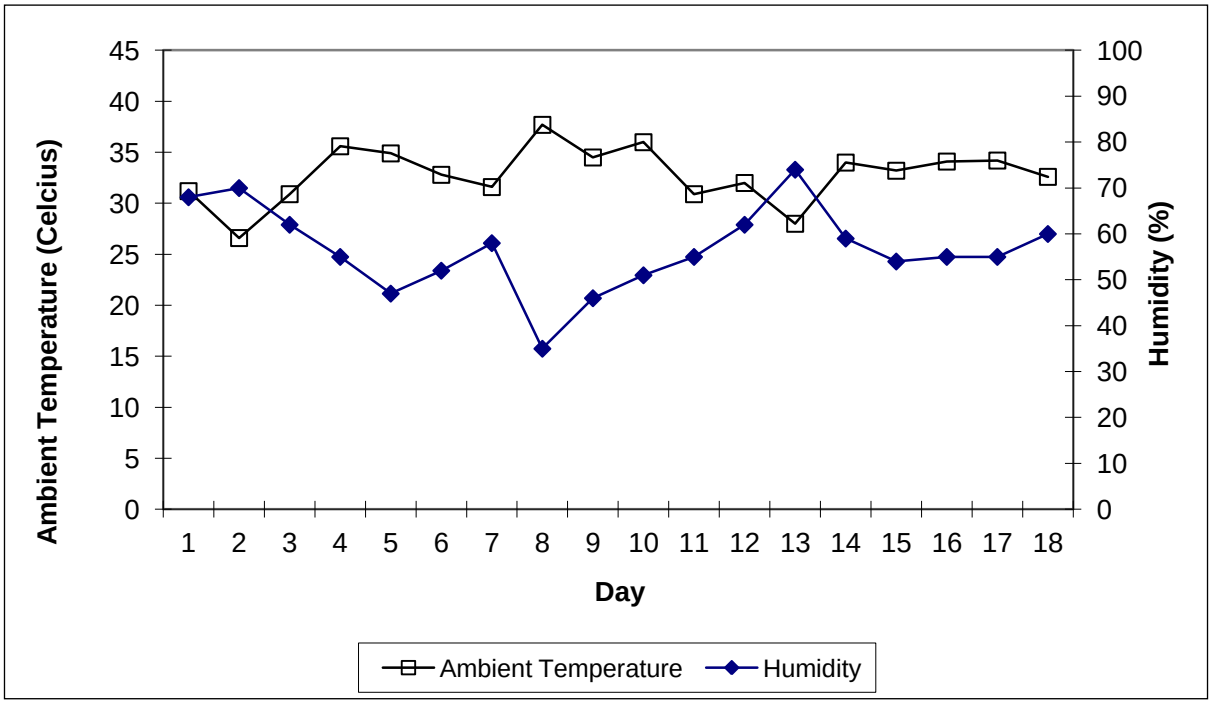

Figure 4.20. Indoor ambient temperature and humidity in the wooden hut during Study 6 conducted in Tanjong Sepat, Selangor from 6 – 23 May 2008 (18 Days).

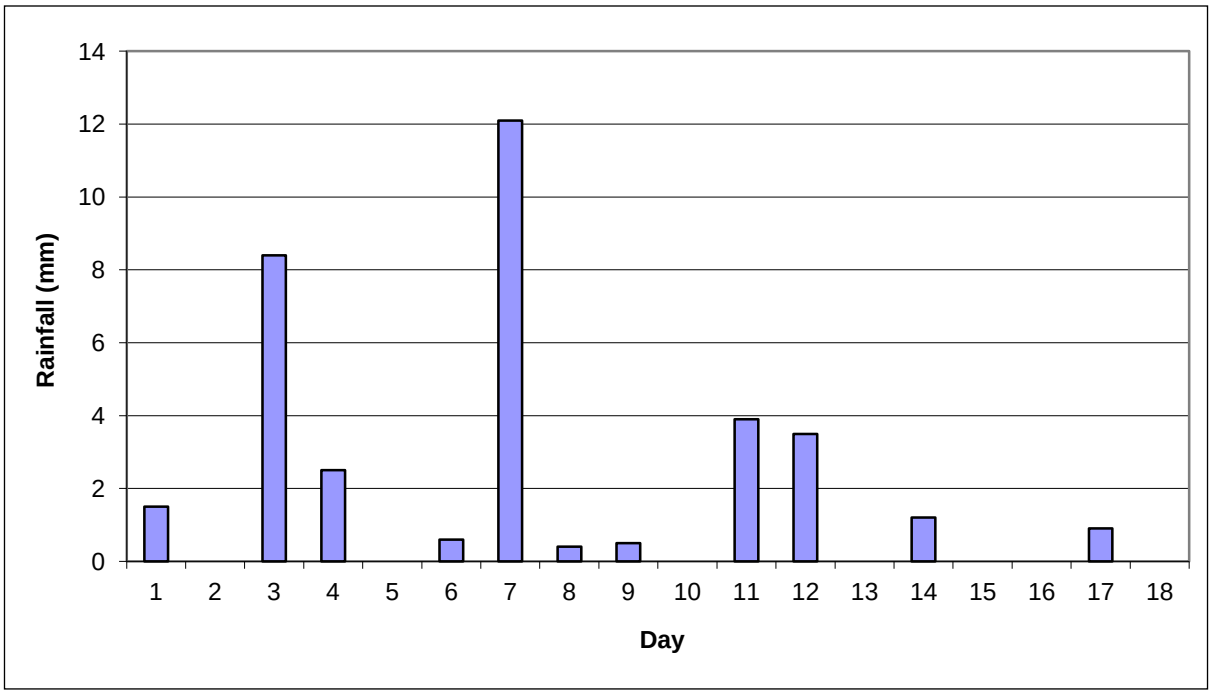

Figure 4.21. Daily rainfall amount in Tanjong Sepat, Selangor during Study 6 conducted from 6 – 23 May 2008 (18 Days). Data was provided by the Department of Meteorology Malaysia.

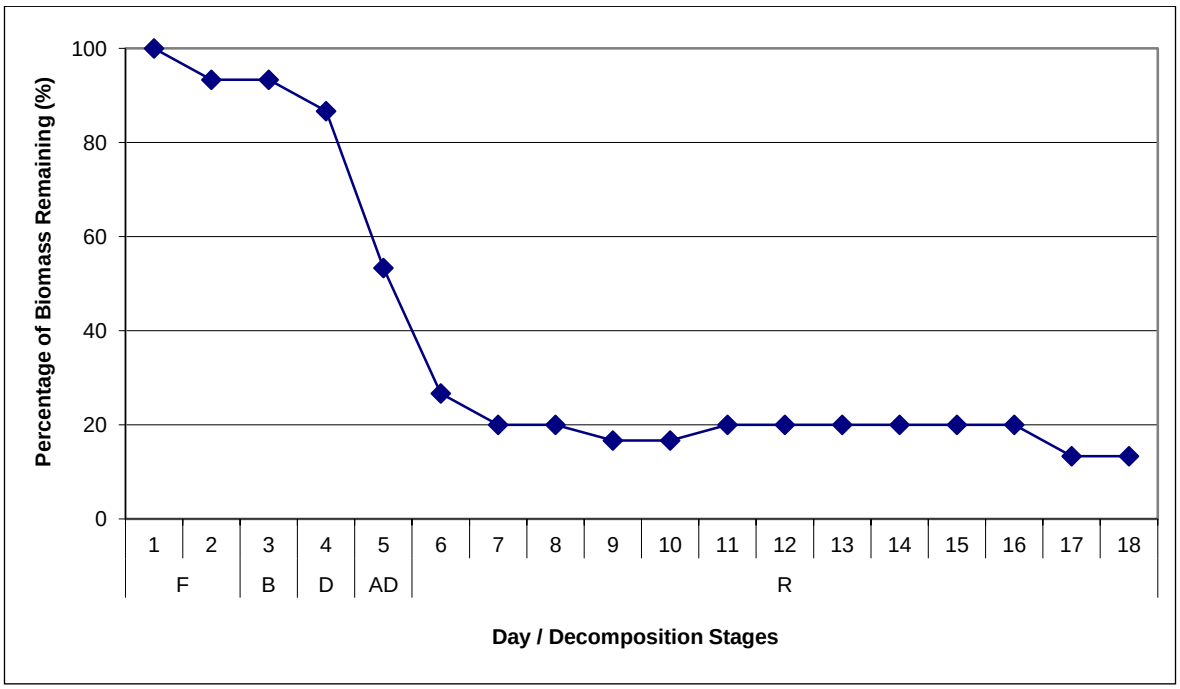

Figure 4.22. Percentage of biomass remaining of the monkey carcass in Study 6 placed in coastal area located in Tanjong Sepat, Selangor. The decomposition of carcass in Study 6 had undergone 5 stages, namely fresh (F), bloating (B), decay (D), advanced decay (AD) and remains (R).

Study 8 commenced simultaneously with Study 7 (serve as a control) on 22 July 2008 at 1100h and completed on 5 August 2008 (time frame of 15 days). The ambient temperature, relative humidity and rainfall during the Study 8 are shown in Figure 4.23 and Figure 4.24, respectively. The ambient temperature ranged from 26.0 to 35.0ºC (mean 31.05 ± 0.55ºC) and relative humidity ranged from 38 to 76% (mean 62.07 ± 2.45%). The Department of Meteorology Malaysia recorded 11 rainy days within 15 days of study period that accounted daily rainfall amount ranging from 0.1 to 10.9mm (mean 4.80 ± 1.06mm).

The decomposition of the carcass in Study 8 had undergone 5 stages namely, fresh (Day 1 – 2), bloating (Day 3 – 4), decay (Day 5), advanced decay (Day 6) and remains (Day 7 – 15). The percentage of biomass remaining of the monkey carcass was shown in Figure 4.25. The decomposition process caused decrease of body weight,

leaving only 32.43% of total body weight in the end of Study 8. The entomofauna obtained from monkey carcass in Study 8 is shown in Table 4.10.

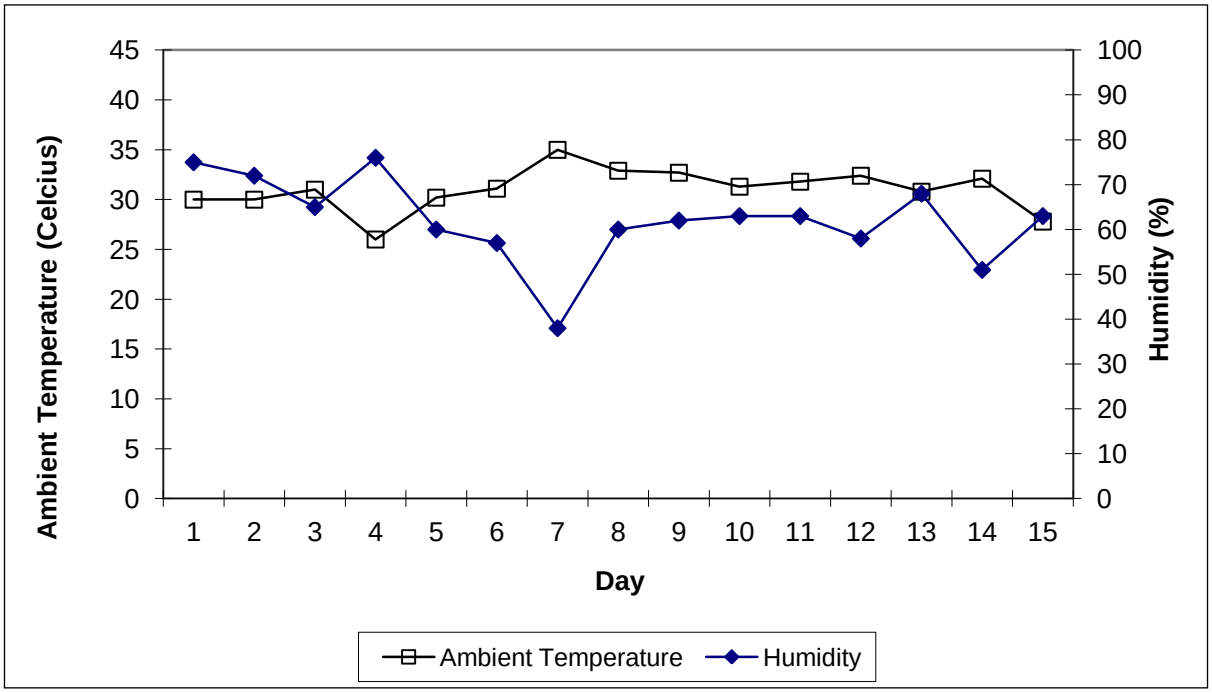

Figure 4.23. Indoor ambient temperature and humidity in the wooden hut during Study 8 conducted in Tanjong Sepat, Selangor from 22 July – 5 August 2008 (15 Days).

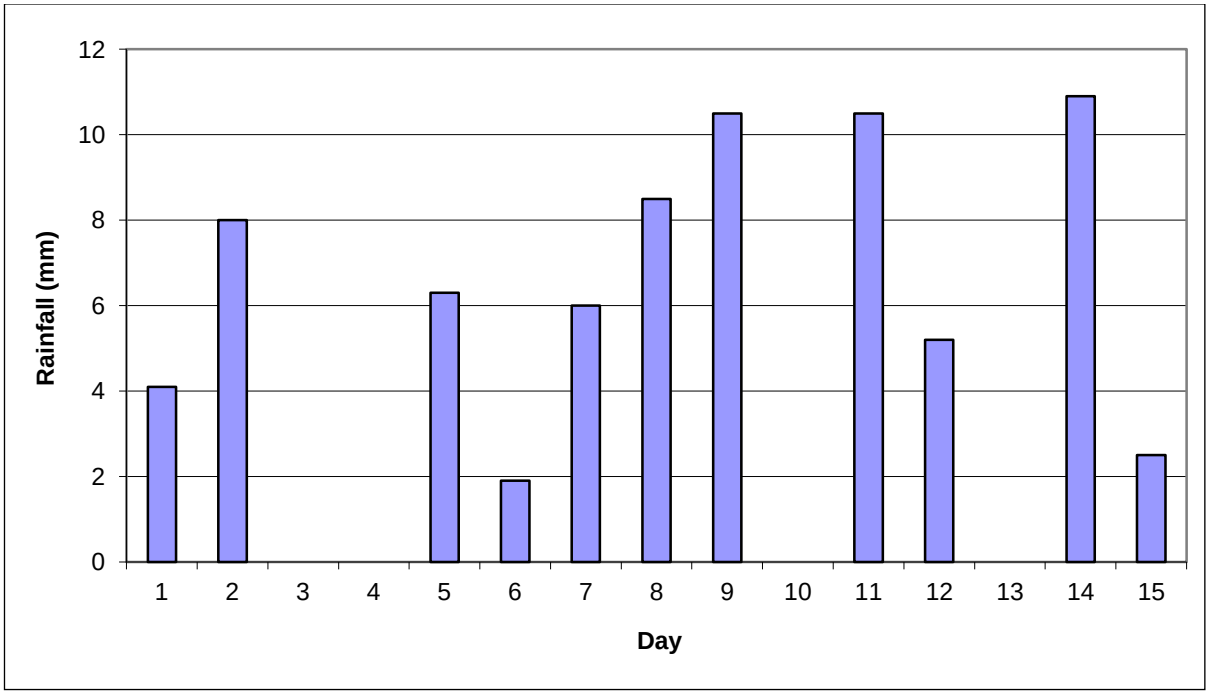

Figure 4.24. Daily rainfall amount in Tanjong Sepat, Selangor during Study 8 conducted from 22 July – 5 August 2008 (15 Days). Data was provided by the Department of Meteorology Malaysia.

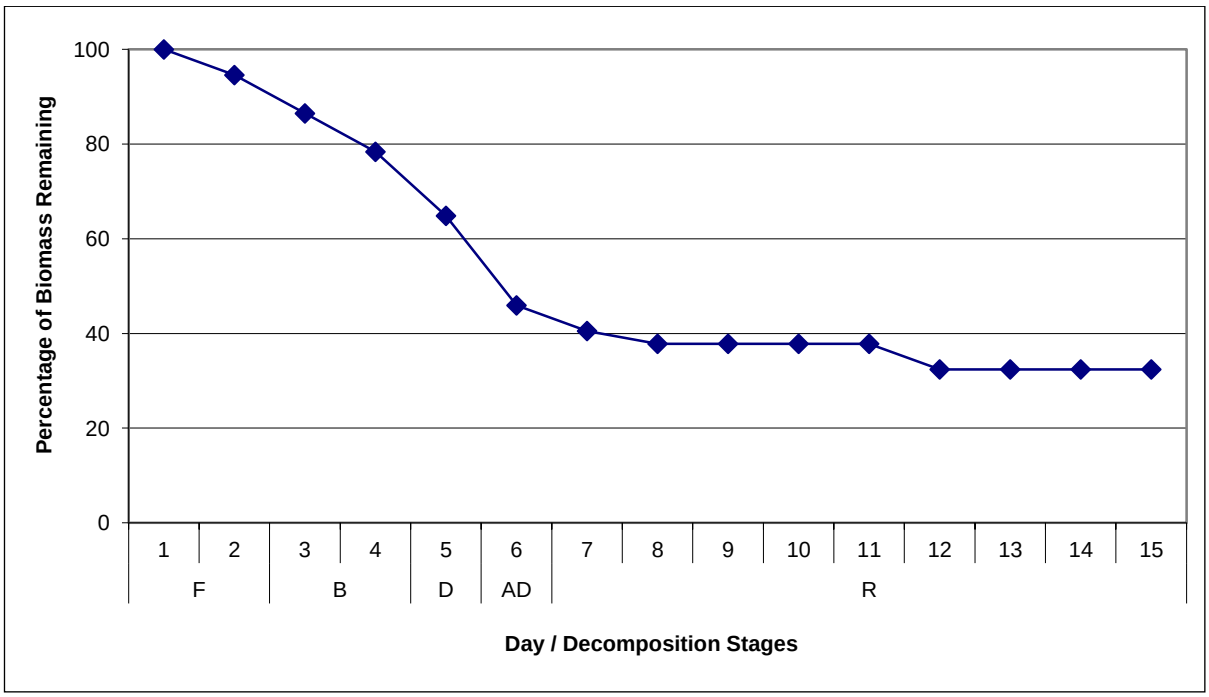

Figure 4.25. Percentage of biomass remaining of monkey carcass in Study 8 placed in coastal area located in Tanjong Sepat, Selangor. The decomposition of carcass in Study 8 had undergone 5 stages, namely fresh (F), bloating (B), decay (D), advanced decay (AD) and remains (R).

Fresh stage (Study 6: Day 1 – 2 / Study 8: Day 1 – 2). After the monkey was euthanised and placed indoor at 1100h, ants [*Paratrechina longicornis* (Hymenoptera: Formicidae: Formicinae)] were observed as the first arthropod to visit the carcass in both studies with 30 minutes. The ants were attracted to the bloodstain on the floor from the wound on the head region. No fly and no oviposition were observed on Day 1.

On Day 2, no fly and no oviposition were observed on the monkey carcass in Study 6. However, flies belonging to the families of Calliphoridae (*C. megacephala* and *C. rufifacies*), Muscidae (*Musca domestica*), Phoridae and Sarcophagidae (*Sarcophaga* spp.) were seen resting inside the wooden hut in Study 8. Eggs were observed at the mouth region. Fresh stage for both studies lasted for 2 days.

Bloating stage (Study 6: Day 3 / Study 8: Day 3 – 4). The stomach of monkey carcasses was bloated on Day 3 in both studies. However, no odour was present. In Study 6, flies (*C. megacephala*, *C. rufifacies*, *M. domestica* and *Sarcophaga* spp.) were

present inside the wooden hut on Day 3, and eggs were seen at the head region. However, no larvae were found until the end of this stage, while in Study 8, adults of *C. megacephala*, *C. rufifacies*, *Hempyrelia* spp., *M. domestica*, *Ophyra spinigera*, phorid and sarcophagid were seen inside the hut, but only 1st-, 2nd- and 3rd-instar larvae of *C. megacephala* were found on the head region throughout the bloating stage. Ants were seen actively predating the fly larvae. The bloating stage in Study 6 and Study 8 lasted for 1 day and 2 days, respectively.

Decay stage (Study 6: Day 4 / Study 8: Day 5). In the decay stage, hairs began to detach, and the carcasses were deflated due to the gases escaping from stomach. Strong odour was present at this stage. Maggots masses consisting of 2nd-instar larvae of *C. megacephala*, *C. rufifacies* and *Sarcophaga* spp. were collected from the head and shoulder regions in Study 6; while 3rd- and 2nd-instar larvae of *C. megacephala*, *C. rufifacies* and *Hemipyrelia* spp. were collected from the head, legs and anus regions in Study 8. Ants were seen predating the fly larvae actively. The decay stage in both studies lasted for a day.

Advanced decay stage (Study 6: Day 5 / Study 8: Day 6). The hairs of carcasses were totally detached and skin layer dehydrated. The number of flies present inside the hut was reduced obviously. No adult fly was seen in Study 6, and only few (less than 10) *M. domestica* and phorids were seen in Study 8. However, maggot masses consisting of 3rd-instar larvae of *C. megacephala*, *C. rufifacies* and *Hemipyrelia* spp. were seen feeding on the head and abdomen regions. Post feeding *C. megacephala* and *C. rufifacies* larvae begun to migrate away from the carcasses for pupation. The advanced decay stage in both studies lasted for a day.

Remains stage (Study 6: Day 6 – 18 / Study 8: Day 7 – 15). The remains stage begun from Day 6 and Day 7 in Study 6 and Study 8, respectively. This stage was noticed when remains were mummified. No adult calliphorid was seen visiting the

carcasses, only phorids and *M. domestica* were seen visiting the carcasses throughout the remains stage. Only larvae of *C. rufifacies* were collected from Day 7 onwards until the end of the studies. Pupae of *C. rufifacies* and *C. megacephala* were collected around the carcasses and around the hut on Day 8 in both studies. Newly emerged adult of *C. rufifacies* and *C. megacephala* were collected on Day 12 and Day 11 in Study 6 and Study 8, respectively. Ants were seen carrying away the fly larvae and pupae, and predating on newly emerged flies. Both studies ended on Day 18 and Day 15, respectively, when there was no larva found on the carcasses on the following day.

Table 4.9. Entomofauna attracted to various decomposition stages of monkey carcass (Study 6) placed in indoor of coastal area located in Tanjong Sepat, Selangor.

Decomposition stages (Duration, Day)	Order	Family	Species	Stage
Fresh (Day 1 – 2)	Hymenoptera	Formicidae	*Paratrechina longicornis*	A
Bloating (Day 3)	Diptera	Calliphoridae	*Chrysomya megacephala*	A, E
			Chrysomya rufifacies	A, E
		Muscidae	*Musca domestica*	A
		Sarcophagidae	*unidentified sp.*	A
	Hymenoptera	Formicidae	*Paratrechina longicornis*	A
Decay (Day 4)	Diptera	Calliphoridae	*Chrysomya megacephala*	A, L2
			Chrysomya rufifacies	A, L2
		Muscidae	*Musca domestica*	A
		Phoridae	*unidentified sp.*	A
		Sarcophagidae	*unidentified sp.*	A, L2
	Hymenoptera	Formicidae	*Paratrechina longicornis*	A
Advanced Decay (Day 5)	Diptera	Calliphoridae	*Chrysomya megacephala*	L3
			Chrysomya rufifacies	L3
	Hymenoptera	Formicidae	*Paratrechina longicornis*	A
Dry Remains (Day 6 – 18)	Diptera	Calliphoridae	*Chrysomya megacephala*	L3
			Chrysomya rufifacies	L3, P, NE
		Muscidae	*Musca domestica*	A
	Hymenoptera	Formicidae	*Paratrechina longicornis*	A

A = adult; L1 = 1st instar larvae; L2 = 2nd instar larvae; L3 = 3rd instar larvae; P = pupae; NE = newly emerged adult

Table 4.10. Entomofauna attracted to various decomposition stages of monkey carcass (Study 8) placed in indoor of coastal area located in Tanjong Sepat, Selangor.

Decomposition stages (Duration, Day)	Order	Family	Species	Stage
Fresh (Day 1 – 2)	Diptera	Calliphoridae	*Chrysomya megacephala*	A, E
			Chrysomya rufifacies	A
		Muscidae	*Musca domestica*	A
		Phoridae	*unidentified sp.*	A
		Sarcophagidae	*unidentified sp.*	A
	Hymenoptera	Formicidae	*Paratrechina longicornis*	A
Bloating (Day 3 – 4)	Diptera	Calliphoridae	*Chrysomya megacephala*	A, L1, L2, L3
			Chrysomya rufifacies	A
			Hemipyrelia sp.	A
		Muscidae	*Musca domestica*	A
			Ophyra spinigera	A
		Phoridae	*unidentified sp.*	A
		Sarcophagidae	*unidentified sp.*	A
	Hymenoptera	Formicidae	*Paratrechina longicornis*	A
Decay (Day 5)	Diptera	Calliphoridae	*Chrysomya megacephala*	L3
			Chrysomya rufifacies	L3
			Hemipyrelia sp.	L2
		Muscidae	*Musca domestica*	A
		Phoridae	*unidentified sp.*	A
	Hymenoptera	Formicidae	*Paratrechina longicornis*	A
Advanced Decay (Day 6)	Diptera	Calliphoridae	*Chrysomya megacephala*	L3
			Chrysomya rufifacies	L3
			Hemipyrelia sp.	L3
		Muscidae	*Musca domestica*	A
		Phoridae	*unidentified sp.*	A
	Hymenoptera	Formicidae	*Paratrechina longicornis*	A
Dry Remains (Day 7 – 15)	Diptera	Calliphoridae	*Chrysomya megacephala*	P, NE
			Chrysomya rufifacies	L3, P, NE
		Muscidae	*Musca domestica*	A
		Phoridae	*unidentified sp.*	A
	Hymenoptera	Formicidae	*Paratrechina longicornis*	A

A = adult; L1 = 1st instar larvae; L2 = 2nd instar larvae; L3 = 3rd instar larvae; P = pupae; NE = newly emerged adult

4.3 BUKIT CINCIN (MONTANE FORESTED AREA)

A total of four studies were conducted in montane forested area located in Bukit Cincin, Pahang. Study 9 and Study 11 (Replicate 1 and Replicate 2) were designed to study the decomposition process and arthropod succession on the monkey carcass placed in outdoor (serves as control); and for comparison purpose, Study 10 and Study 12 (Replicate 1 and Replicate 2) were designed to study the decomposition process and arthropod succession on the monkey carcass placed in indoor. Study 9 (Outdoor, Replicate 1) and Study 10 (Indoor, Replicate 1) were conducted in the same study site in which they were only separated by 100 meters. Similar setup was also done on Study 11 and Study 12. Furthermore, Study 11 (Outdoor, Replicate 2) and Study 12 (Indoor, Replicate 2) were conducted in a site that separated by 2 km from Study 9 and Study 10.

Due to absence of fly activity during nocturnal period from previous studies, thus all studies conducted in montane forested area commenced during the diurnal period only. Hence, four studies were conducted simultaneously from 26 May 2009 until 9 July 2009.

4.3.1 STUDY 9 AND STUDY 11

DECOMPOSITION PROCESS AND ARTHROPOD SUCCESSION ON MONKEY CARCASS IN OUTDOOR (REPLICATE 1 AND 2)

Study 9 and Study 11 were done simultaneously on daytime from 26 May 2009 until 7 July 2009. Both studies were conducted on sites and separated by a distance of 2 km. There was no significant difference in the mean ambient temperature and relative humidity (Table 4.11) between both studies. In addition, both sites had the same daily

rainfall data during the course of study. Thus, the results of both studies were combined, analysed and explained in this section.

Table 4.11. Comparison on mean outdoor ambient temperature, relative humidity and daily rainfall amount between forensic entomological studies (Study 9 and Study 11) conducted in montane forested area of Bukin Cincin, Pahang.

Meteorology	Study 9 (Replicate 1) 26 May – 7 July 2009	Study 11 (Replicate 2) 26 May – 7 July 2009	t-test
Temperature, ºC	20.99 ± 0.36	21.05 ± 0.30	P = 0.899, t = -0.128, df = 62
Humidity, %	69.72 ± 2.32	73.00 ± 2.29	P = 0.318, t = -1.006, df = 62
Rainfall, mm	2.91 ± 1.13	2.91 ± 1.13	Not Applicable

$p > 0.05$: not significantly difference $p \leq 0.05$: significantly difference

The ambient temperature, relative humidity and daily rainfall during the forensic entomological studies conducted in outdoor located in Bukit Cincin, Pahang are shown in Figure 4.26 and Figure 4.27, respectively. The ambient temperature for both studies ranged from 18.0 to 26.8ºC (mean 20.99 ± 0.36ºC) and 18.0 to 26.0ºC (mean 21.73 ± 0.30ºC), respectively. On the other hand, relative humidity in both studies ranged from 43 to 93% (mean 69.72 ± 2.32%) and 46 to 95% (mean 73.00 ± 2.29%), respectively. The Department of Meteorology Malaysia recorded 12 rainy days within 43 days of study period that accounted for daily rainfall ranging from 0.5 to 32.0 mm (mean 2.91 ± 1.13 mm).

As in other sites, 5 decomposition stages, namely, fresh, bloating, decay, advanced decay and remains were also observed in Study 9 and 11. The percentage of biomass remaining of the monkey carcasses was shown in Figure 4.28 and Figure 4.29. The decomposition process caused decrease of body weight, leaving only 21.88% and 11.36% of total body weight at the end of both studies, respectively. The entomofauna obtained from monkey carcasses in Study 9 and Study 11 are shown in Table 4.12 and Table 4.13.

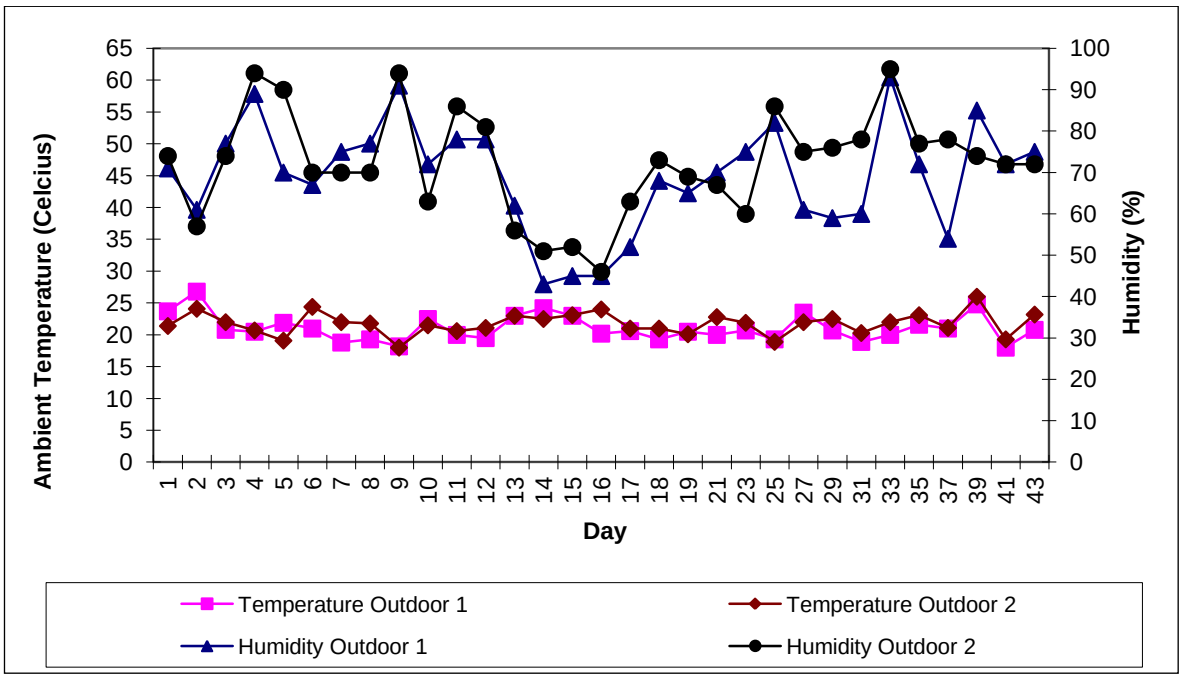

Figure 4.26. Ambient temperature and relative humidity of the study sites during Study 9 (Outdoor 1) and Study 11 (Outdoor 2) conducted in Bukit Cincin, Pahang from 26 May – 7 July 2009 (43 Days).

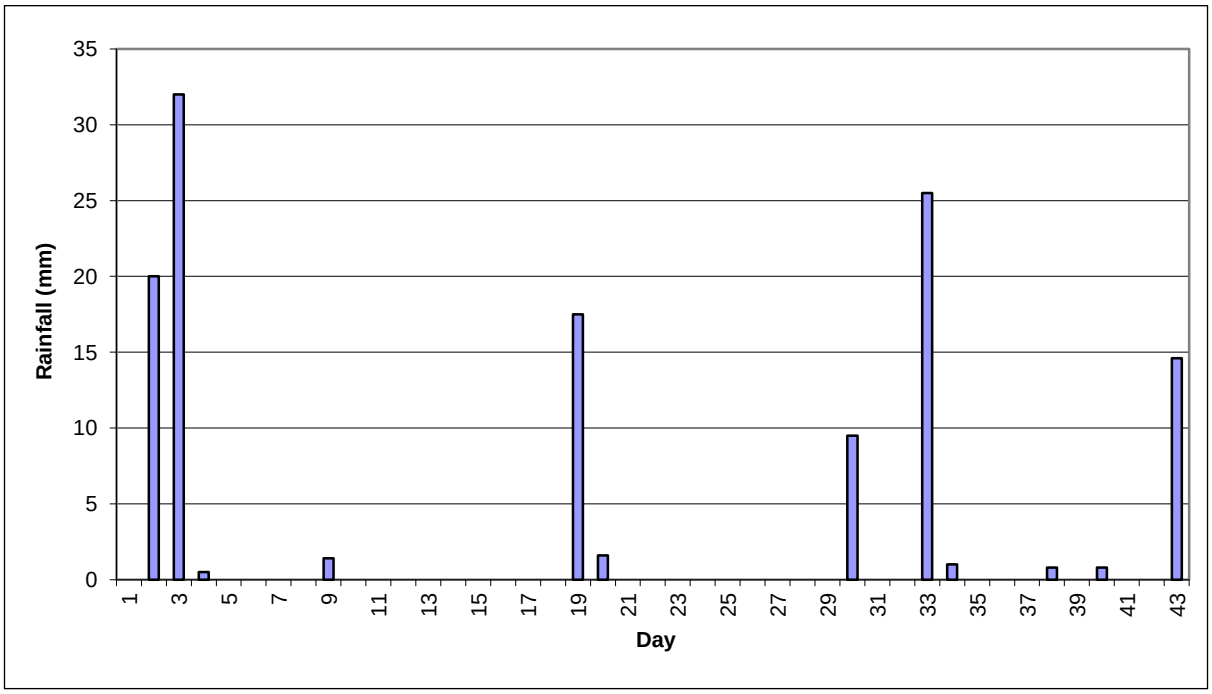

Figure 4.27. Daily rainfall amount in Bukit Cincin, Pahang during Study 9 (Outdoor 1) and Sudy 11 (Outdoor 2) conducted from 26 May – 7 July 2009 (43 Days). Data was provided by the Department of Meteorology Malaysia.

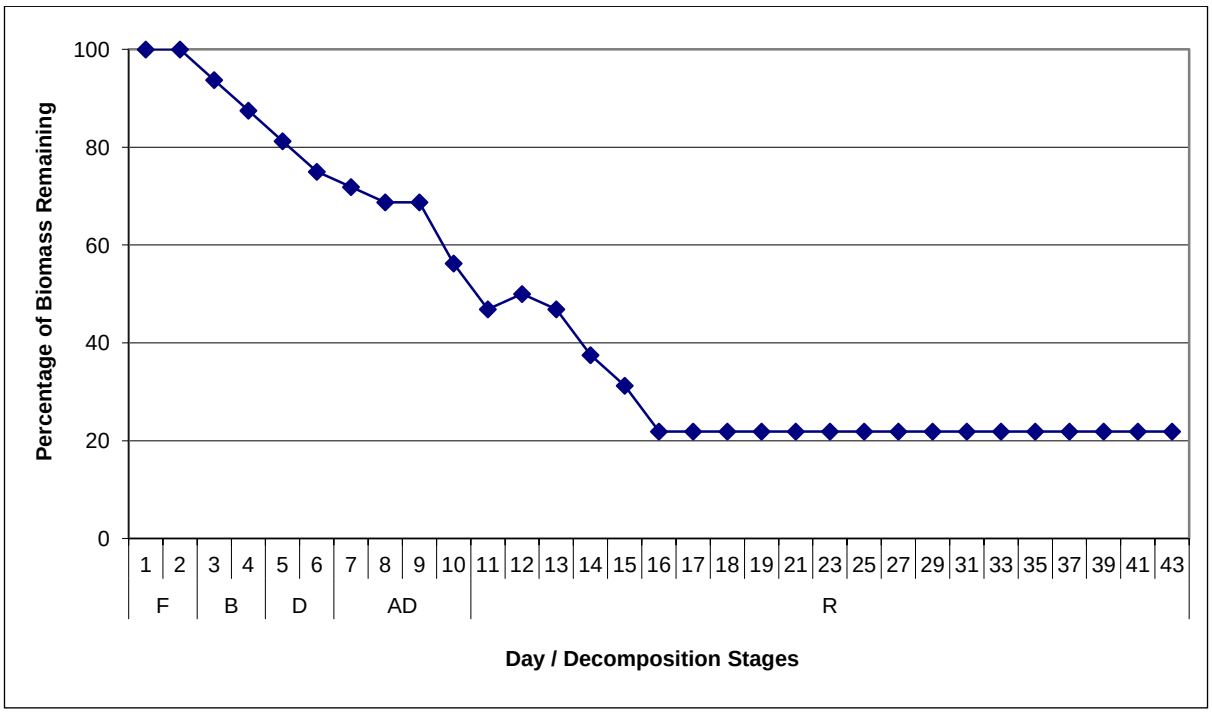

Figure 4.28. Percentage of biomass remaining of monkey carcass in Study 9 (Outdoor 1) placed in montane forested area located in Bukit Cincin, Pahang. The decomposition of carcass in Study 9 had undergone 5 stages namely, fresh (F), bloating (B), decay (D), advanced decay (AD) and remains (R).

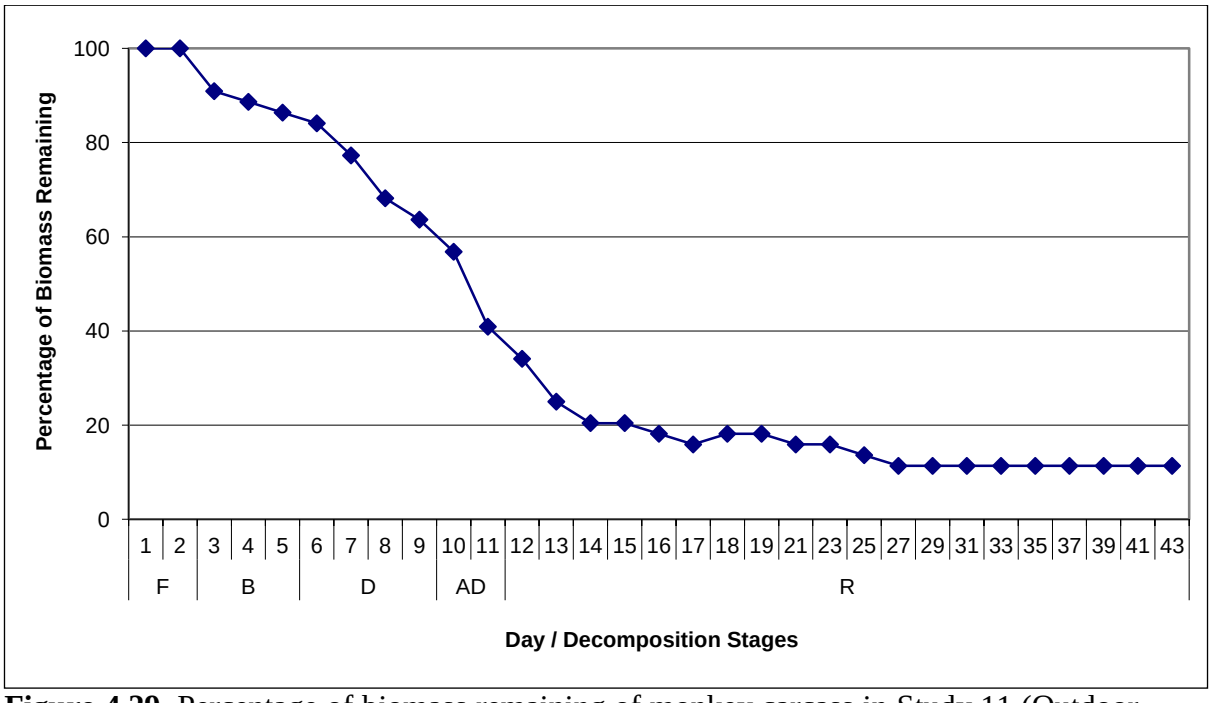

Figure 4.29. Percentage of biomass remaining of monkey carcass in Study 11 (Outdoor 2) placed in montane forested area located in Bukit Cincin, Pahang. The decomposition of carcass in Study 11 had undergone 5 stages namely, fresh (F), bloating (B), decay (D), advanced decay (AD) and remains (R).

Fresh stage (Study 9: Day 1 – 2 / Study 11: Day 1 – 2). The monkeys were euthanised on the same day at 1100h (Study 9) and 1200h (Study 11), respectively. Study 11 was conducted 1 hour later after Study 9.

In Study 9, *Hypopygiopsis violacea* was the first visitor attracted to the monkey carcass within 30 minutes, followed by *Chrysomya megacephala*, *Chrysomya villeneuve* and *Lucilia porphyrina*. Flies were observed to lay eggs at the mouth region. No ants were observed on the first day. On Day 2, fly egg masses were found on the neck and arm regions of carcass. First-instar larvae of *Chrysomya villeneuve* and *Lucilia porphyrina* were collected from the neck region. However, no *Hypopygiopsis violacea* larvae were collected from the carcass. The number of adult flies increased (to about 30 flies) on Day 2.

In Study 11, the experiment was conducted an hour later after Study 9. Due to the rain, no flies and ants were observed visiting the carcass on Day 1. On Day 2, adults of *Chrysomya megacephala*, *Chrysomya pinguis*, *Chrysomya villeneuve*, *Lucilia porphyrina*, *Tabanus* spp. and sarcophagid were seen visiting the carcass. First-instar larvae of *Hypopygiopsis violacea* were colletced from the mouth region.

Ants (Hymenoptera: Formicidea), *Paratrechina longicornis* and *Pheidole longipes* were seen to predate the fly eggs and larvae in both studies. The fresh stage in both studies lasted for 2 days.

Bloating stage (Study 9: Day 3 – 4 / Study 11: Day 3 – 5). The bloating stage was noticed once the abdomen began to bloat due to accumulated gases produced by bacteria within the intestine or stomach. The larvae of *H. violacea* (2nd- and 3rd-instar), *C. villeneuve* (1st- and 2nd-instar), *C. pinguis* (1st- and 2nd-instar), *Lucilia porphyrina* (2nd- and 3rd-instar) and sarcophagid (1st-, 2nd- and 3rd-instar) were collected from mouth, neck, ears, armpit and anus regions at this stage. Bettles, *Creochara brevipennis* (Coleoptera: Staphylinidae) were seen predating the maggot masses, while wasp

(Hemenoptera: Vespidae) were seen to carry away the larvae. The bloating stage lasted for 2 days and 3 days in Study 9 and 11, respectively.

Decay stage (Study 9: Day 5 – 6 / Study 11: Day 6 – 9). This stage was noticed when the stomach contents were exposed and the abdomen was deflated. The number of adult blowflies which visited the carcasses in this stage decreased; while *Ophyra spinigera* adults increased. Maggot masses consisted of 2nd- and 3rd-instar of *C. villeneuve*, *C. pinguis*, *Lucilia porphyrina* and sarcophagid which were seen on the head and stomach regions. Second-instar larvae of *O. spinigera* were collected at the end of this stage. The number of bettles [Hydrophilidae (*Sphaeridium* spp.), Silphidae (*Chrysosilpha formosa*) and Staphylinidae (*Creochara brevipennis* and *Philonthus* spp.)] that predated on the fly larvae increased. Because the maggots were actively consuming the fly larvae, the percentage of biomass decreased rapidly. A butterfly, *Vagrans egista macromalayana* (Lepidoptera: Nymphalidae) was seen visiting the carcass in Study 11. The decay stage lasted for 2 days and 4 days for Study 9 and Study 11, respectively.

Advanced decay stage (Study 9: Day 7 – 10 / Study 11: Day 10 – 11). Advanced decay stage was noticed when majority of the bones and cartilages were observed. Majority of the flesh and inner organs were consumend by fly larvae. The odour of decaying materials begun to fade away.

In Study 9, larvae of *Chrysomya* spp. were seen to migrate from the carcass for pupation on Day 7, and the pupae of *C. pinguis* and *C. villeneuve* were collected on Day 8 at the bushes around the carcass. A butterfly was seen to visit the carcass on Day 10, while in Study 11, the carcass began dehydration and pupae of *C. pinguis* were found on the carcass on Day 10. The carcass was mummified on Day 11. Second-instar larvae of *O. spinigera* were found under both carcasses.

Three species of ants (*Pachycondyla* sp., *Paratrechina longicornis* and *Pheidole longipes*) and wasps were seen carrying away the fly larvae and pupae. Butterflies, *Kaniska canace perakana* (Lepidoptera: Nymphalidae), *Udara toxopeusi toxopeusi* (Lepidoptera: Lycaenidae) and *Udara placidula irenae* (Lepidoptera: Lycaenidae) were seen to visit the carcasses in both studies. Cockroaches were also seen attracted to the carcass. Advanced decay stage lasted for 4 days and 2 days, respectively.

Remains stage (Study 9: Day 11 – 43 / Study 11: Day 12 – 43). The remains stage of both carcasses was noticed once the carcasses were totally mummified. Less odour was noticed in this stage. The percentage of biomass remaining decreased slowly until it stabilized. Larvae of *C. pinguis* and *C. villeneuve* were only found in the beginning of this stage, and slowly being replaced by *O. spinigera* until the end of both studies. Newly emerged adult flies (*C. pinguis* and *C. villeneuve*) were noticed on Day 14 (Study 9) and Day 15 (Study 11). In Study 9, larvae of *Fannia canicularis* and *Fannia scalaris* were seen on Day 18 onwards until end of study, while in Study 11, larvae of *Fannia cancularis* were only collected on Day 29. The predators of fly larvae, pupae and newly emerged adults increased in this stage, such as beetles [Coleoptera: Hydrophilidae (*Cercyon* spp.), Silphidae (*Chrysosilpha formosa*) and Staphylinidae (*Aleochara* spp., *Atheta* spp., *Creochara brevipennis*, *Hesperus* spp. and *Philonthus* spp.)], ants [Hymenoptera: Formicidae (*Pachycondyla* spp., *Paratrechina longicornis* and *Pheidole longipes*)], wasp [Hymenoptera: Vespidae], bees [Hymenoptera: Apidae], grasshoppers [Orthoptera: Tetrigidae], earwigs [Dermptera] and cockroaches [Blattodae]. Newly emerged adult flies of *O. spinigera* were noticed on Day 41 in both studies. Both studies were terminated on Day 43 when no more larvae were observed on following day. The remains stage took about 33 days and 32 days for Study 9 and Study 11, respectively.

Table 4.12. Entomofauna attracted to various decomposition stages of monkey carcass (Study 9) placed in outdoor of montane forested area located in Bukit Cincin, Pahang.

Decomposition stages (Duration, Day)	Order	Family	Species	Stage
Fresh (Day 1 – 2)	Diptera	Calliphoridae	*Chrysomya megacephala*	A
			Chrysomya pinguis	A
			Chrysomya villeneuve	A, L1
			Hypopygiopsis violacea	A
			Lucilia porphyrina	A, L1
		Sarcophagidae	*unidentified sp.*	A
	Hymenoptera	Formicidae	*Paratrechina longicornis*	A
			Pheidole longipes	A
Bloating (Day 3 – 4)	Diptera	Calliphoridae	*Chrysomya megacephala*	A
			Chrysomya pinguis	A, L1, L2
			Chrysomya villeneuve	A, L2
			Lucilia porphyrina	A, L2, L3
		Sarcophagidae	*unidentified sp.*	A
	Hymenoptera	Formicidae	*Paratrechina longicornis*	A
			Pheidole longipes	A
	Coleoptera	Staphylinidae	*Creochara brevipennis*	A
Decay (Day 5 – 6)	Diptera	Calliphoridae	*Chrysomya megacephala*	A
			Chrysomya pinguis	A, L2, L3
			Chrysomya villeneuve	A, L3
			Lucilia porphyrina	A, L3
		Muscidae	*Ophyra spinigera*	A
	Hymenoptera	Formicidae	*Pachycondyla sp.*	A
			Paratrechina longicornis	A
			Pheidole longipes	A
	Coleoptera	Staphylinidae	*Creochara brevipennis*	A
			Philonthus sp.	A
Advanced Decay (Day 7 – 10)	Diptera	Calliphoridae	*Chrysomya megacephala*	A
			Chrysomya pinguis	L3, P
			Chrysomya villeneuve	A, L3, P
			Lucilia porphyrina	A
		Muscidae	*Ophyra spinigera*	A, L2
	Hymenoptera	Formicidae	*Pachycondyla sp.*	A
			Paratrechina longicornis	A
			Pheidole longipes	A
	Coleoptera	Elateridae	*unidentified sp.*	A
		Silphidae	*Chrysosilpha formosa*	A
		Staphylinidae	*Creochara brevipennis*	A
			Philonthus sp.	A
			Ontholestes sp.	A
	Lepidoptera	Nymphalidae	*Kaniska canace perakana*	A
	Blattodea	Unidentified	*unidentified sp.*	A
Dry Remains (Day 11 – 43)	Diptera	Calliphoridae	*Chrysomya megacephala*	A
			Chrysomya pinguis	L3, P, NE
			Chrysomya villeneuve	A, L3, P, NE
			Lucilia porphyrina	A,
		Muscidae	*Ophyra spinigera*	A, L3, P, NE
		Fanniidae	*Fannia canicularis*	L3
			Fannia scalaris	L3
	Hymenoptera	Formicidae	*Hypoponera sp.*	A
			Pachycondyla sp.	A
			Paratrechina longicornis	A
			Pheidole longipes	A
		Vespidae	*unidentified sp.*	A
	Coleoptera	Hydrophilidae	*Cercyon sp.*	A
		Silphidae	*Chrysosilpha formosa*	A, L
		Staphylinidae	*Atheta sp.*	A
			Creochara brevipennis	A
			Hesperus sp.	A
			Philonthus sp.	A
	Lepidoptera	Lycaenidae	*Udara placidula irenae*	A
	Dermaptera	Unidentified	*unidentified sp.*	A

A = adult; L = Larvae; L1 = 1st instar larvae; L2 = 2nd instar larvae; L3 = 3rd instar larvae; P = pupae; NE = newly emerged adult

Table 4.13. Entomofauna attracted to various decomposition stages of monkey carcass (Study 11) placed in outdoor of montane forested area located in Bukit Cincin, Pahang.

Decomposition stages (Duration, Day)	Order	Family	Species	Stage
Fresh (Day 1 – 2)	Diptera	Calliphoridae	*Chrysomya megacephala*	A
			Chrysomya pinguis	A
			Chrysomya villeneuve	A
			Hypopygiopsis violacea	L1
			Lucilia porphyrina	A
		Sarcophagidae	*unidentified sp.*	A
		Tabanidae	*Tabanus sp.*	A
	Hymenoptera	Formicidae	*Paratrechina longicornis*	A
			Pheidole longipes	A
Bloating (Day 3 – 5)	Diptera	Calliphoridae	*Chrysomya megacephala*	A
			Chrysomya pinguis	A, L1, L2
			Chrysomya villeneuve	A, L1, L2
			Hypopygiopsis violacea	L2, L3
			Lucilia porphyrina	A
		Sarcophagidae	*unidentified sp.*	L1, L2, L3
	Hymenoptera	Formicidae	*Paratrechina longicornis*	A
			Pheidole longipes	A
		Vespidae	*unidentified sp.*	A
	Coleoptera	Staphylinidae	*Creochara brevipennis*	A
Decay (Day 6 – 9)	Diptera	Calliphoridae	*Chrysomya megacephala*	A
			Chrysomya pinguis	A, L3
			Chrysomya villeneuve	A, L2, L3
			Hypopygiopsis violacea	L3
			Lucilia porphyrina	A, L2, L3
			Stomorhina xanthogaster	A
		Muscidae	*Ophyra spinigera*	A, L2
		Sarcophagidae	*unidentified sp.*	L3
	Hymenoptera	Formicidae	*Paratrechina longicornis*	A
			Pheidole longipes	A
		Vespidae	*unidentified sp.*	A
	Coleoptera	Hydrophilidae	*Sphaeridium sp.*	A
		Silphidae	*Chrysosilpha formosa*	A
		Staphylinidae	*Creochara brevipennis*	A
	Lepidoptera	Nymphalidae	*Vagrans egista macromalayana*	A
Advanced Decay (Day 9 – 11)	Diptera	Calliphoridae	*Chrysomya megacephala*	A
			Chrysomya pinguis	A, L3, P
			Chrysomya villeneuve	A, L3
			Lucilia porphyrina	A
		Muscidae	*Musca domestica*	A
			Ophyra spinigera	A, L2
		Syrphidae	*Eristalis solitus (= E. cerealis)*	A
	Hymenoptera	Formicidae	*Paratrechina longicornis*	A
			Pheidole longipes	A
			Pachycondyla sp.	A
		Vespidae	*unidentified sp.*	A
	Coleoptera	Silphidae	*Chrysosilpha formosa*	A
		Staphylinidae	*Creochara brevipennis*	A
	Lepidoptera	Lycaenidae	*Udara toxopeusi taxopeusi*	A
			Udara placidula irenae	A
Dry Remains (Day 12 – 43)	Diptera	Calliphoridae	*Chrysomya megacephala*	A
			Chrysomya pinguis	A, L3, P, NE
			Chrysomya villeneuve	A, L3, P, NE
			Stomorhina xanthogaster	A
		Muscidae	*Ophyra spinigera*	A, L3, P, NE
		Fanniidae	*Fannia canicularis*	L3
	Hymenoptera	Formicidae	*Paratrechina longicornis*	A
			Pheidole longipes	A
			Pachycondyla sp.	A
		Apidae	*unidentified sp.*	A
		Vespidae	*unidentified sp.*	A
	Coleoptera	Silphidae	*Chrysosilpha formosa*	A, L
		Staphylinidae	*Aleochara*	A
			Creochara brevipennis	A
			Philonthus sp.	A, L
	Orthoptera	Tetrigidae	*unidentified sp.*	A
	Blattodea	Unidentified	*unidentified sp.*	A

A = adult; L = Larvae; L1 = 1st instar larvae; L2 = 2nd instar larvae; L3 = 3rd instar larvae; P = pupae; NE = newly emerged adult

4.3.2 STUDY 10 AND STUDY 12

DECOMPOSITION PROCESS AND ARTHROPOD SUCCESSION ON MONKEY CARCASS IN INDOOR (REPLICATE 1 AND REPLICATE 2)

Study 10 and Study 12 commenced simultaneously on daytime from 26 May 2009 until 9 July 2009. Both studies were conducted on sites and separated by a distance of 2 km. There was no significant difference between mean ambient temperature, relative humidity and daily rainfall amount (Table 4.14) recorded in both studies during the course of study. Thus, the observation and results of both studies were combined, analysed and explained in this section.

Table 4.14. Comparison on mean indoor ambient temperature, relative humidity and daily rainfall amount between forensic entomological studies (Study 10 and Study 12) conducted in a wooden hut (indoor) located in montane forested area of Bukit Cincin, Pahang.

Meteorology	Study 10 (Replicate 1) 26 May – 5 July 2009	Study 12 (Replicate 2) 26 May – 9 July 2009	t-test
Temperature, ºC	20.78 ± 0.40	20.90 ± 0.25	P = 0.797, t = -0.258, df = 60
Humidity, %	69.53 ± 1.87	72.50 ± 2.05	P = 0.291, t = -1.006, df = 60
Rainfall, mm	2.70 ± 1.15	2.78 ± 1.09	P = 0.960, t = -0.051, df = 84

$p > 0.05$: not significantly difference $p \leq 0.05$: significantly difference

The indoor ambient temperature, relative humidity and daily rainfall amount during the forensic entomological studies conducted in wooden huts located in Bukit Cincin, Pahang are shown in Figure 4.30 and Figure 4.31, respectively. The indoor ambient temperature for both studies ranged from 17.8 to 26.9ºC (mean 20.78 ± 0.40ºC) and 17.2 to 22.8ºC (mean 19.90 ± 0.25ºC), respectively. On the other hand, relative humidity inside both wooden huts ranged from 50 to 91% (mean 69.53 ± 1.87%) and 46 to 95% (mean 72.50 ± 2.05%), respectively. According to the Department of Meteorology Malaysia, Study 10 recorded 11 rainy days within 41 days of study period

(mean daily rainfall amount = 2.70 ± 1.15mm); while Study 12 recorded 12 rainy days within 45 days of study period (mean daily rainfall = 2.78 ± 1.09mm).

The decomposition of carcasses in Study 10 and Study 12 had undergone 5 stages namely, fresh, bloating, decay, advanced decay and remains. The percentage of biomass remaining of monkey carcasses are shown in Figure 4.32 and Figure 4.33. The decomposition process caused decrease of body weight, leaving only 26.67% and 27.78% of total body weight at the end of both studies, respectively. The entomofauna obtained from monkey carcasses in Study 10 and Study 12 are shown in Table 4.15 and Table 4.16.

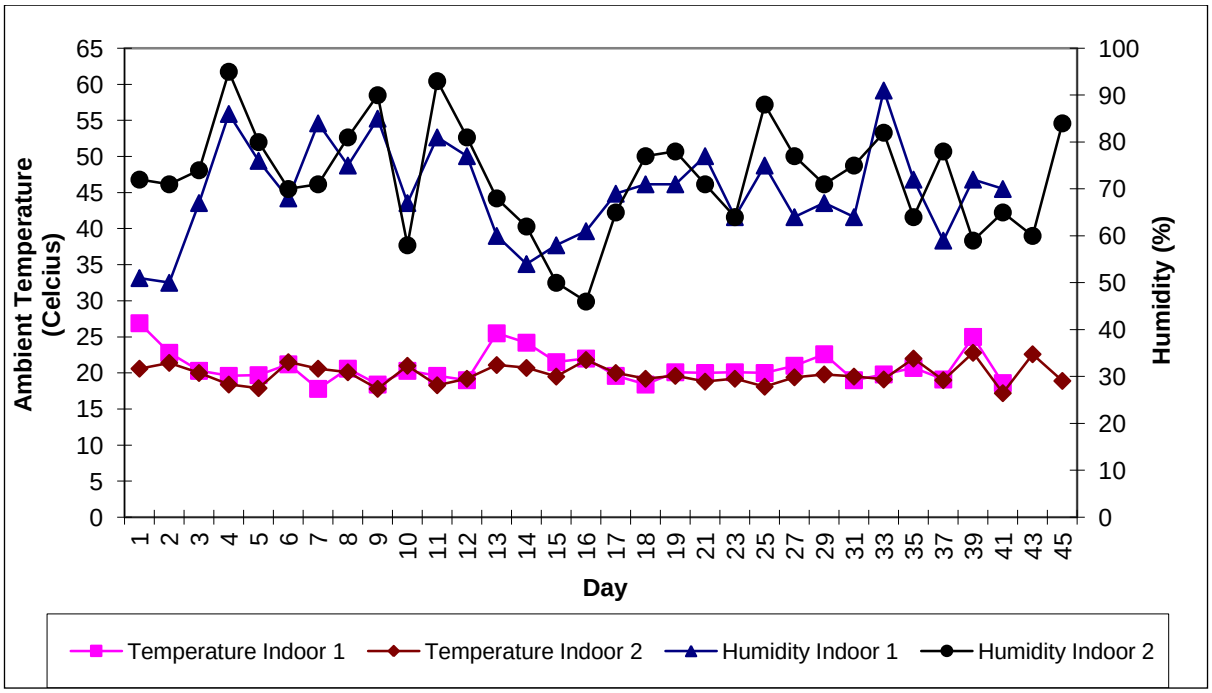

Figure 4.30. Indoor ambient temperature and humidity in the wooden hut during Study 10 and Study 12 conducted in Bukit Cincin, Pahang from 26 May – 9 July 2009 (45 Days).

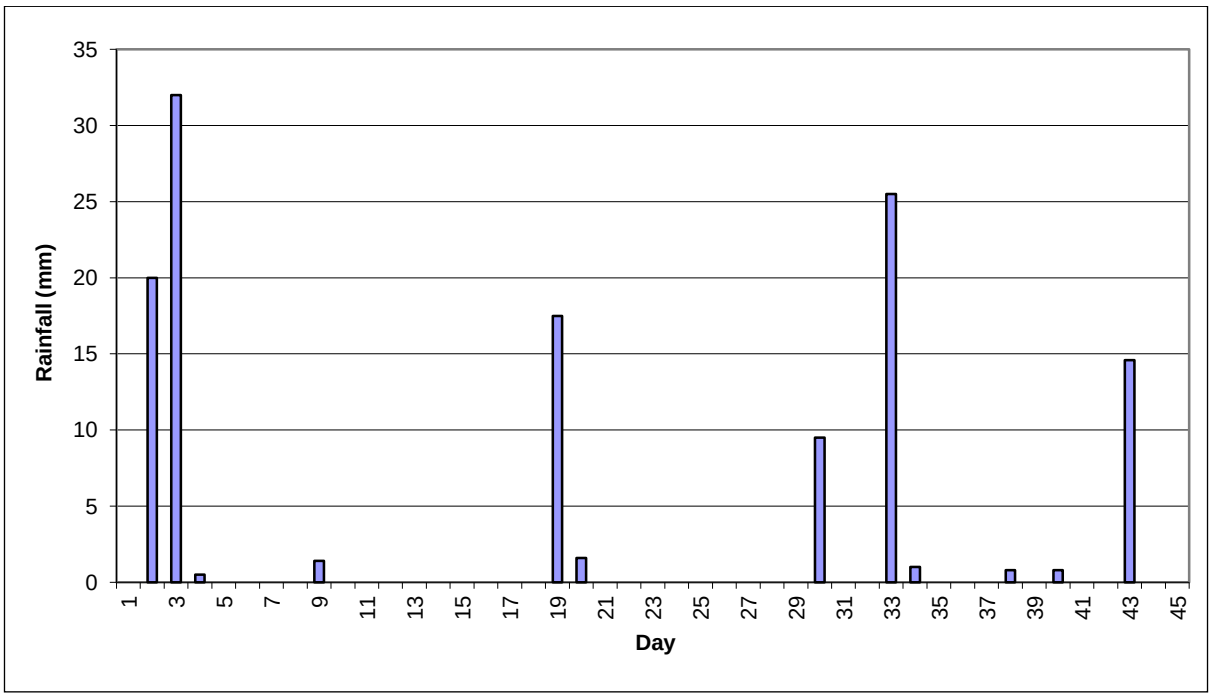

Figure 4.31. Daily rainfall amount in Bukit Cincin, Pahang during Study 10 and Study 12 conducted from 26 May – 9 July 2009 (45 Days). Data was provided by the Department of Meteorology Malaysia.

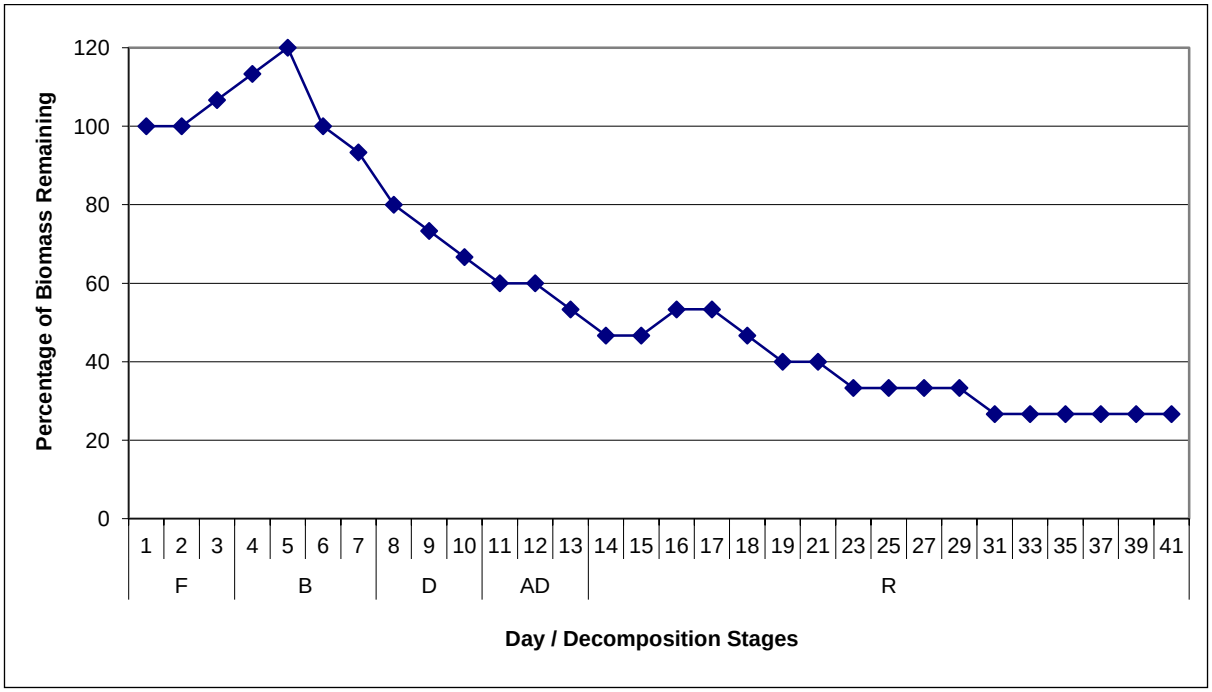

Figure 4.32. Percentage of biomass remaining of monkey carcass in Study 10 placed indoor in montane forested area located in Bukit Cincin, Pahang. The decomposition of carcass in Study 10 had undergone 5 stages, namely fresh (F), bloating (B), decay (D), advanced decay (AD) and remains (R).

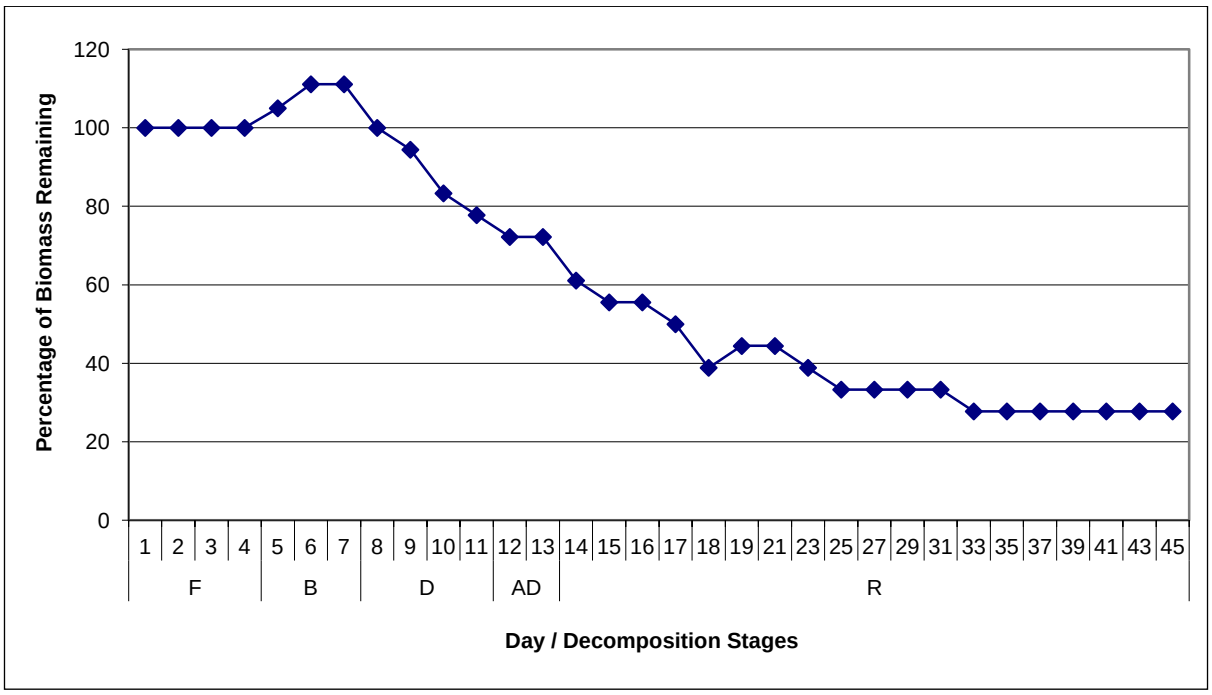

Figure 4.33. Percentage of biomass remaining of monkey carcass in Study 12 placed indoor in montane forested area located in Bukit Cincin, Pahang. The decomposition of carcass in Study 12 had undergone 5 stages, namely fresh (F), bloating (B), decay (D), advanced decay (AD) and remains (R).

Fresh stage (Study 10: Day 1 – 3 / Study 12: Day 1 – 4). The monkeys were euthanised on the same day at 1130h (Study 10) and 1230h (Study 12), respectively. Study 12 commenced 1 hour later than Study 10. In Study 10, no insect visitor was observed on the carcass on Day 1. Only ants, *Pheidole longipes* (Hymenoptera: Formicidae) were observed to visit the carcass on Day 2 and Day 3. No fly visited the carcass throughout the fresh stage. On the other hand, no insects were observed visiting carcass in Study 12 throughout the fresh stage. The fresh stage in Study 10 and Study 12 lasted for 3 days and 4 days, respectively.

Bloating stage (Study 10: Day 4 – 7 / Study 12: Day 5 – 7). In Study 10, a *Hypopygiopsis violacea* was seen visiting the carcass on Day 4. Eggs and 1st-instar larvae of *H. violacea* were collected from the anus region on the same day. On Day 5, less than 10 flies consisted of *Chrysomya megacephala*, *Chrysomya pinguis*, *Chrysomya villeneuve* and *Lucilia porphyrina* were seen inside the wooden hut. Second-

instar larvae of *H. violacea* were collected. On Day 6 and Day 7, larvae of *H. violacea* (3rd-instar), *C. pinguis* (1st-instar), *C. villeneuve* (1st- and 2nd-instar) and *L. porphyrina* (1st- and 2nd-instar) were collected from head, chest, legs and stomach regions. Beetles [*Sericania* spp. (Coleoptera: Scarabaeidae), *Creochara brevipennis* (Coleoptera: Staphylinidae)], cockroach [Blattodae] and ants [*Pheidole longipes* (Hymenoptera: Formicidae)] were seen predating on the fly eggs and larvae.

In Study 12, no fly oviposition was noticed in Day 5. On Day 6, approximately 11 flies consisted of *C. megacephala* and *H. violacea* were seen inside the wooden huts. These flies were seen ovipositing their eggs actively on the mouth and ear regions. A cockroach [Blattodae] and spider [Arachnida: Araneae] was seen predating on the fly eggs and adult flies, respectively. On Day 7, 1st- and 2nd-instar larvae of *C. megacephala*, *H. violacea* and *Sarcophaga* spp. were collected. The number of fly attracted to the carcass increased (about 30 flies). Adults of *C. megacephala*, *C. pinguis*, *C. villeneuve*, *L. porphyrina* and *Ophyra spinigera* were collected at the end of bloating stage. In addition, beetles (*Atheta* spp. and *Creochara brevipennis*) belonging to the family Staphylinidae was seen predating the fly larvae. No ants were observed visiting the carcass.

The bloating stage in Study 10 and Study 12 lasted for 4 Days and 3 Days, respectively.

Decay stage (Study 10: Day 8 – 10 / Study 12: Day 8 – 11). Decay stage of both studies begun on Day 8. Approximately 80 flies were observed inside the wooden hut. In Study 10, larvae of *C. pinguis* (2nd- and 3rd-instar), *C. villeneuve* (2nd- and 3rd-instar) and *L. porphyrina* (3rd-instar) were collected from maggot masses on the head and stomach regions. In Study 12, larvae of *C. megacephala* (2nd- and 3rd-instar), *C. pinguis* (2nd- and 3rd-instar), *C. villeneuve* (2nd- and 3rd-instar), *H. violacea* (2nd- and 3rd-instar), *L. porphyrina* (2nd-instar) and *Sarcophaga* spp. (3rd-instar) were also

collected from maggot masses on the head, anus and stomach regions. Beetles (Coleoptera: Chrysomelidae, Curculionidae, Staphylinidae), ants (Hymenoptera: Formicidae), wasps (Hymenoptera: Vespidae), Cockroaches (Blattode) and Centipede (Chilopoda) were seen visiting the carcasses throughout the decay stage. The decay stage lasted for 3 days and 4 days in Study 10 and Study 12, respectively.

Advanced decay stage (Study 10: Day 11 – 13 / Study 12: Day 12 – 13). The number of calliphorid adults attracted to the carcasses decreased; while number of muscid adults (*Ophyra spinigera*) increased. Both carcasses begun to mummify on Day 12. Larvae of calliphorid were seen migrating from the carcasses looking for a drier place for pupation. On Day 13, pupae of *C. megacephala*, *C. pinguis* and *C. villeneuve* were collected around the carcasses and at the corner of the wooden hut. Second and third-instar larvae of *O. spinigera* were dominant in the maggot masses. The advanced decay stage ended once the carcasses were totally mummified. Advanced decay stage lasted for 3 days and 2 days in Study 10 and Study 12, respectively.

Remains stage (Study 10: Day 14 – 45 / Study 12: Day 14 – 41). The remains stage of both carcasses was noticed on Day 14. Only 3rd-instar larvae of calliphorids were collected on the early remains stage (from Day 14 – Day 25). Newly emerged adults of *C. megacephala*, *C. pinguis* and *C. villeneuve* were observed on Day 17 and Day 18 in Study 10 and Study 12, respectively. Ants, bees, wasps, beetles, cockroaches and spiders were seen predating on the larvae, pupae and newly emerged adults. From Day 25 onwards, only 3rd-instar larvae of *O. spinigera* were collected until end on studies. Newly emerged adults of *O. spinigera* were observed on Day 31 and Day 27 in Study 10 and Study 12, respectively. The remains stage took about 28 days and 30 days for Study 10 and Study 12, respectively.

Table 4.15. Entomofauna attracted to various decomposition stages of monkey carcass (Study 10) placed in indoor of montane forested area located in Bukit Cincin, Pahang.

Decomposition stages (Duration, Day)	Order	Family	Species	Stage
Fresh (Day 1 – 3)	Hymenoptera	Formicidae	*Pheidole longipes*	A
Bloating (Day 4 – 7)	Diptera	Calliphoridae	*Chrysomya megacephala*	A
			Chrysomya pinguis	A, L1
			Chrysomya villeneuve	A, L1, L2
			Hypopygiopsis violacea	A, E, L1, L2, L3
			Lucilia porphyrina	A, L1, L2
	Hymenoptera	Formicidae	*Pheidole longipes*	A
	Coleoptera	Scarabaeidae	*Sericania sp.*	A
		Staphylinidae	*Creochara brevipennis*	A
	Blattodae	Unidentified	*unidentified sp.*	A
Decay (Day 8 – 10)	Diptera	Calliphoridae	*Chrysomya megacephala*	A
			Chrysomya pinguis	A, L2, L3
			Chrysomya villeneuve	A, L2, L3
			Lucilia porphyrina	A, L3
		Muscidae	*Ophyra spinigera*	A
	Hymenoptera	Formicidae	*Pheidole longipes*	A
	Coleoptera	Staphylinidae	*Creochara brevipennis*	A
Advanced Decay (Day 11 – 13)	Diptera	Calliphoridae	*Chrysomya megacephala*	A
			Chrysomya pinguis	A, L3, P
			Chrysomya villeneuve	A, L2, L3, P
			Lucilia porphyrina	A
		Muscidae	*Ophyra spinigera*	A, L2, L3
	Hymenoptera	Formicidae	*Pheidole longipes*	A
	Coleoptera	Staphylinidae	*Creochara brevipennis*	A
Dry Remains (Day 14 – 41)	Diptera	Calliphoridae	*Chrysomya megacephala*	A
			Chrysomya pinguis	A, L3, P, NE
			Chrysomya villeneuve	A, L3, P, NE
		Muscidae	*Ophyra spinigera*	A, L3, P, NE
		Fanniidae	*Fannia canicularis*	L3
		Syrphidae	*Eristalis solitus (= E. cerealis)*	A
	Hymenoptera	Formicidae	*Pheidole longipes*	A
		Apidae	*unidentified sp.*	A
	Coleoptera	Elateridae	*unidentified sp.*	A
		Leiodidae	*Nemadus sp.*	A
		Staphylinidae	*Atheta sp.*	A
			Creochara brevipennis	A
	Lepidoptera	Lycaenidae	*Udara placidula irenae*	A
	Blattodae	Unidentified	*unidentified sp.*	A

A = adult; L = Larvae; L1 = 1st instar larvae; L2 = 2nd instar larvae; L3 = 3rd instar larvae; P = pupae; NE = newly emerged adult

Table 4.16. Entomofauna attracted to various decomposition stages of monkey carcass (Study 12) placed in indoor of montane forested area located in Bukit Cincin, Pahang.

Decomposition stages (Duration, Day)	Order	Family	Species	Stage
Fresh (Day 1 – 4)				
Bloating (Day 5 – 7)	Diptera	Calliphoridae	*Chrysomya megacephala*	A, E, L1
			Chrysomya pinguis	A, E
			Chrysomya villeneuve	A, E
			Hypopygiopsis violacea	A, E, L1, L2
			Lucilia porphyrina	A
		Muscidae	*Ophyra spinigera*	A
		Sarcophagidae	*unidentified sp.*	L1, L2
	Coleoptera	Staphylinidae	*Atheta sp.*	A
			Creochara brevipennis	A
	Blattodea	Unidentified	*unidentified sp.*	A
Decay (Day 8 – 11)	Diptera	Calliphoridae	*Chrysomya megacephala*	A, L2, L3
			Chrysomya pinguis	A, L2, L3
			Chrysomya villeneuve	A, L2, L3
			Hypopygiopsis violacea	L2, L3
			Lucilia porphyrina	A, L2
		Muscidae	*Musca domestica*	A
			Ophyra spinigera	A
		Sarcophagidae	*unidentified sp.*	A, L3
	Hymenoptera	Vespidae	*unidentified sp.*	A
	Coleoptera	Chrysomelidae	*unidentified sp.*	A
		Curculionidae	*unidentified sp.*	A
		Staphylinidae	*Creochara brevipennis*	A
			Platydracus sp.	A
	Blattodea	Unidentified	*unidentified sp.*	A
Advanced Decay (Day 12 – 13)	Diptera	Calliphoridae	*Chrysomya megacephala*	A, L3, P
			Chrysomya pinguis	A, L3, P
			Chrysomya villeneuve	A, L3, P
			Hypopygiopsis violacea	L3
			Lucilia porphyrina	A, L3
		Muscidae	*Musca domestica*	A
			Ophyra spinigera	A, L2
		Sarcophagidae	*unidentified sp.*	A, L3
	Hymenoptera	Vespidae	*unidentified sp.*	A
	Coleoptera	Staphylinidae	*Creochara brevipennis*	A
			Philonthus sp.	A
Dry Remains (Day 14 – 45)	Diptera	Calliphoridae	*Chrysomya megacephala*	A, L3, P, NE
			Chrysomya pinguis	L3, P, NE
			Chrysomya villeneuve	A, L3, P, NE
			Lucilia porphyrina	L3
		Phoridae	*unidentified spp.*	A
		Muscidae	*Musca domestica*	A
			Ophyra spinigera	A, L3, P, NE
		Sarcophagidae	*unidentified sp.*	A, L3
	Hymenoptera	Vespidae	*unidentified sp.*	A
	Coleoptera	Elateridae	*unidentified sp.*	A
		Leiodidae	*Nemadus sp.*	A
		Staphylinidae	*Creochara brevipennis*	A
			Philonthus sp.	A
			Platydracus sp.	A

A = adult; L = Larvae; L1 = 1st instar larvae; L2 = 2nd instar larvae; L3 = 3rd instar larvae; P = pupae; NE = newly emerged adult

4.4 THE CIRCADIAN ACTIVITY PATTERN OF BLOWFLIES ON MONKEY CARCASSES

There was no study on the circadian activities of flies associated with monkey remains in this region. The observations on the time when flies began to visit the monkey carcasses in the dawn and left the monkey carcasses in the dusk are shown in Table 4.17 and Table 4.18, respectively.

Study 1 (monkey was euthanised at daytime, 1130h) showed that flies were able to visit the carcass within 30 minutes after the carcass was placed outdoor. On the other hand, Study 3 (monkey was euthanised at nighttime, 2200h) showed that no fly activities throughout the night until the sunrise. Flies were observed to visit the carcass at 0740h and eggs were oviposited by 0800h, indicating that the oviposition behaviour of blow flies was delayed during nighttime.

The result was further confirmed by observation on the fly activities for another 2 consecutive days. Both studies showed that flies visited monkey carcasses about half hour after sunrise (mean 29.25 ± 1.49 minutes, n = 4) (Table 4.17) and actively laid eggs on the carcasses throughout the day until the sunset. No fly was observed visiting the carcasses less than 10 minutes after sunset (mean 5.80 ± 0.68 minutes, n = 5) (Table 4.18). No fly activities were observed throughout the night. Both studies confirmed that flies are inactive at night.

Table 4.17. Observation on time flies began to visit monkey carcasses placed outdoor after sunrise in Ulu Gombak, Selangor.

Monkey carcass	Date / Day	Time		Duration between sunrise and flies began to visit the carcasses (Minute)
		Sunrise[c]	Time flies began to visit the carcass	
Study 1[a]	9 May 2007 / Day 1	0702	No observation	-
	10 May 2007 / Day 2	0702	0728	26
	11 May 2007 / Day 3	0702	0732	30
Study 3[b]	1 Aug 2007 / Day 1	0712	No observation	-
	2 Aug 2007 / Day 2	0712	0740	28
	3 Aug 2007 / Day 3	0712	0745	33
			Mean ± SE (minute)	29.25 ± 1.49

[a]Monkey was euthanised on 9 May 2007, 1130h.
[b]Monkey was euthanised on 1 August 2007, 2200h.
[c]Sunrise time was provided by Department of Meteorology Malaysia.
SE = standard error

Table 4.18. Observation on time when no fly activities were observed on the carcasses placed outdoor after sunset in Ulu Gombak, Selangor.

Monkey carcass	Date / Day	Time		Duration between sunset and no fly activities were observed (Minute)
		Sunset[c]	Time no fly activities were observed	
Study 1[a]	9 May 2007 / Day 1	1917	1925	8
	10 May 2007 / Day 2	1917	1924	7
	11 May 2007 / Day 3	1917	1922	5
Study 3[b]	1 Aug 2007 / Day 1	1927	No observation	-
	2 Aug 2007 / Day 2	1927	1930	3
	3 Aug 2007 / Day 3	1927	1936	6
			Mean ± SE (minute)	5.80 ± 0.86

[a]Monkey was euthanised on 9 May 2007, 1130h.
[b]Monkey was euthanised on 1 August 2007, 2200h.
[c]Sunset time was provided by Department of Meteorology Malaysia.
SE = standard error

4.5 PMI ESTIMATION IN OUTDOOR AND INDOOR CONDITIONS IN DIFFERENT ECOLOGICAL HABITATS

The observation on insect evidence and post mortem interval estimation on monkey carcasses placed in outdoor and indoor in different ecological habitats were presented in Figure 4.34 to Figure 4.36.

Generally, flies immedialtely colonized on the monkeys euthanised at daytime and placed on the ground in outdoor. Thus, no delay on post mortem interval estimation was recorded in all study sites. However, for monkey euthanised at nighttime (Study 3), flies oviposited their eggs on the carcass only after the sunrise on next day. Thus, the post mortem interval estimation was delayed about 12 hours (Figure 4.34).

Flies colonization of the monkey carcasses placed indoor were delayed according to different ecological habitats. At least 3 days delay on PMI estimation was observed on both carcasses placed in wooden hut located in forested area, while only 1 day delay on PMI estimation were observed on both carcasses placed in wooden hut located in coastal area (Figure 4.35). Furthermore, 3 – 4 days delay on PMI estimation was recorded on carcasses placed indoor in montane forested area (Figure 4.36).

This study proved that there was delay on forensically important flies oviposition on carcasses placed in enclosed areas. PMI estimation was not accurate without knowing the conditions of study sites. The occurence of fly to colonise the carcasses found indoor may vary, depending on different ecological habitats.

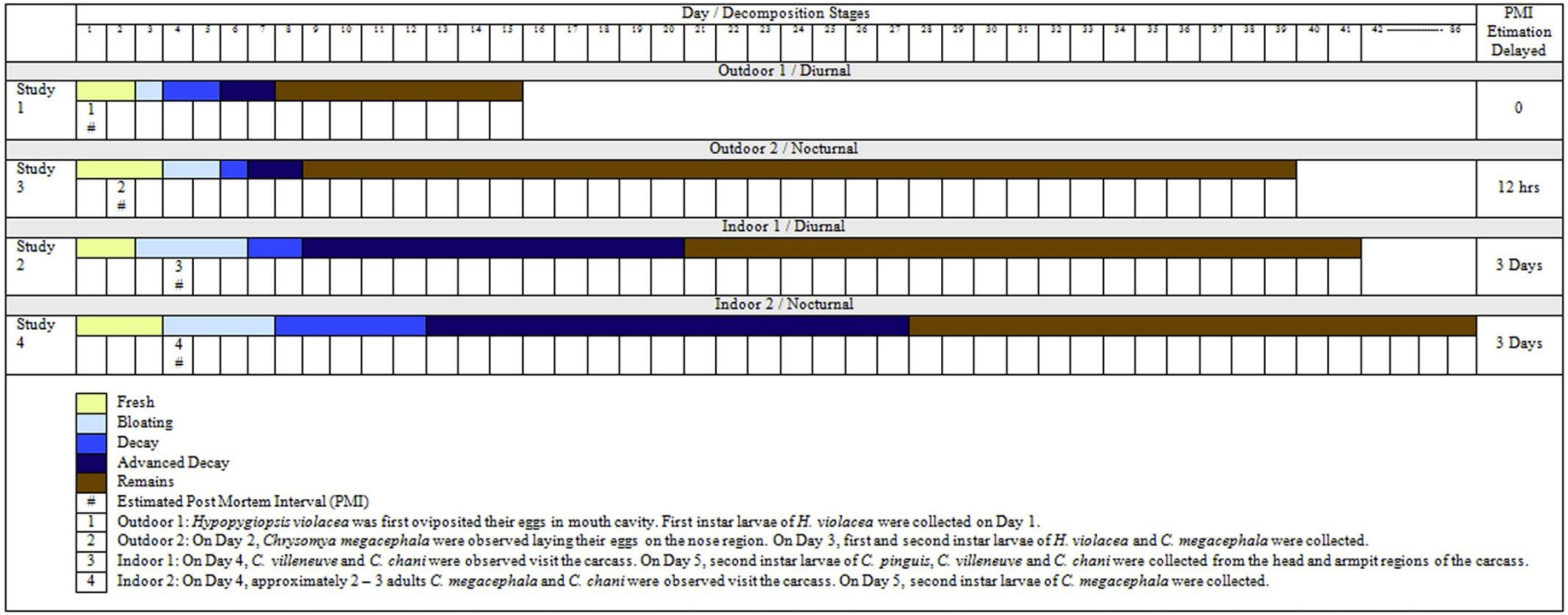

Figure 4.34. Observation on insect evidence and post mortem interval estimation on monkey carcasses placed in outdoor and indoor in forested area, Ulu Gombak, Selangor.

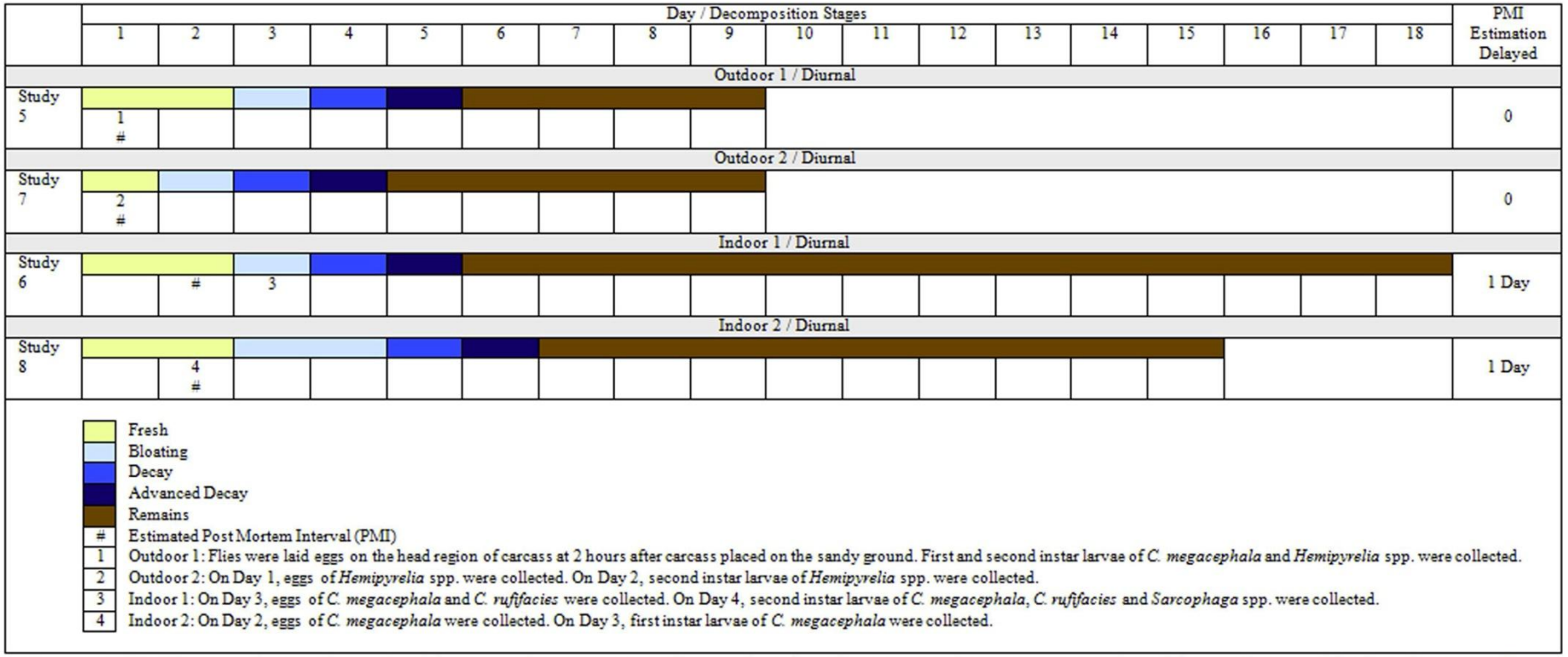

Figure 4.35. Observation on insect evidence and post mortem interval estimation on monkey carcasses placed in outdoor and indoor in coastal area, Tanjong Sepat, Selangor.

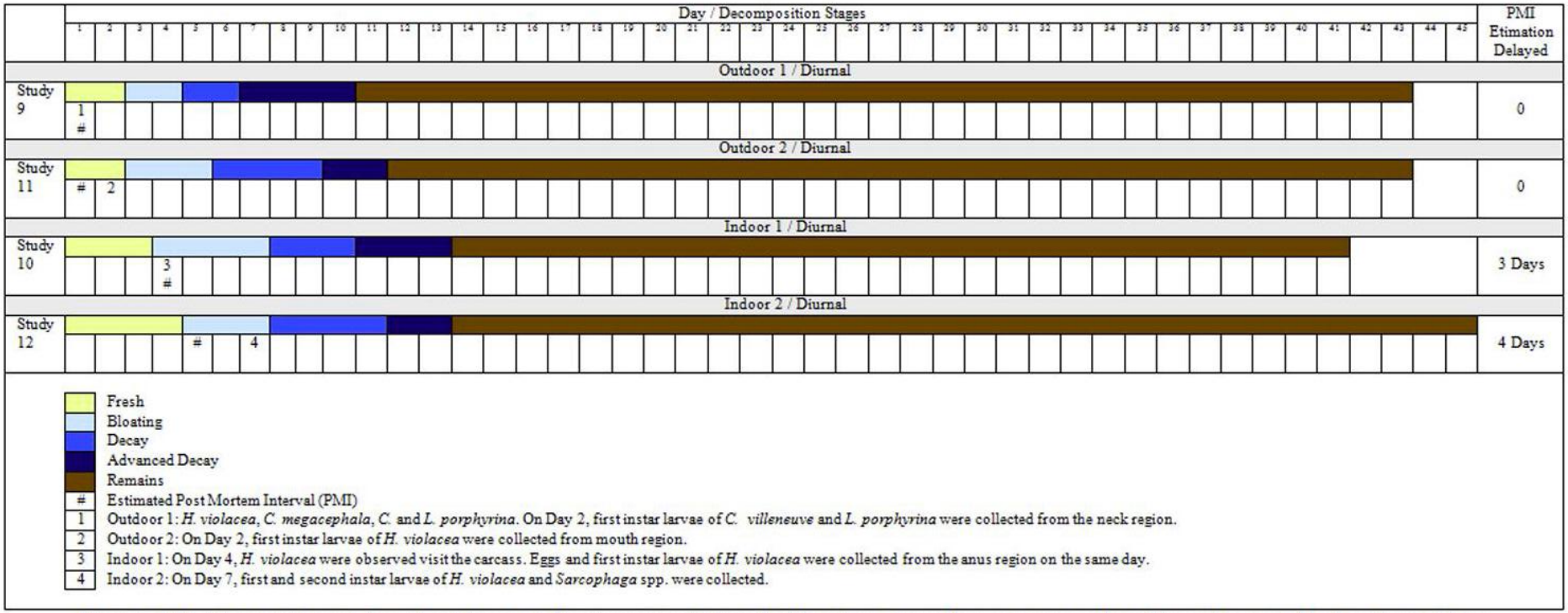

Figure 4.36. Observation on insect evidence and post mortem interval estimation on monkey carcasses placed in outdoor and indoor in montane forested area, Bukit Cincin, Pahang.

4.6 COMPARISON BETWEEN FLIES SUCCESSION AND DECOMPOSITION OF MONKEY CARCASSES IN OUTDOOR AND INDOOR IN DIFFERENT ECOLOGICAL HABITATS

The comparison on mean ambient temperature and relative humidity between forensic entomological studies conducted in outdoor and indoor conditions in different ecological habitats was shown in Table 4.19. There was no significant difference between mean ambient temperature on studies conducted in outdoor and indoor in the same period ($p > 0.05$), indicating that temperature was not playing an significant role on carcasses decomposition located in same ecological habitat at the same period.

On the other hand, relative humidity between outdoor and indoor conditions in coastal area and montane forested area was not significantly different ($p > 0.05$). However, outdoor relative humidity in lowland forested area, Ulu Gombak was significantly higher than indoor (Study 1 > Study 2; and Study 3 > Study 4) ($p < 0.05$). This may due to the density and presence of different kinds of vegetations in different ecological habitats, which are able to maintain the humidity in the atmosphare.

The comparison on mean ambient temperature, relative humidity and rainfall amount in different ecological habitats during the study periods were shown in Table 4.20. Ambient temperature in coastal area (Tanjong Sepat, Selangor) was significantly higher than lowland (Ulu Gombak, Selangor) and montane (Bukit Cincin, Selangor) forested areas ($p < 0.05$), while relative humidity in coastal area was significantly lower than lowland and montane forested areas ($p < 0.05$). However, there was no significant difference between rainfall amount in all study sites during the study period ($p > 0.05$).

Table 4.19. Comparison on mean ambient temperature and relative humidity between forensic entomological studies conducted in outdoor and indoor conditions.

Study Site	Replicate	Outdoor vs Indoor	t-test	
			Temperature	Relative Humidity
Lowland Forested Area (Ulu Gombak, Selangor)	1	Study 1 vs Study 2	P = 0.217 df = 57 t = 1.249	P = 0.000 df = 57 t = 4.095
	2	Study 3 vs Study 4	P = 0.905 df = 77 t = 0.120	P = 0.000 df = 77 t = 5.700
Coastal Area (Tanjong Sepat, Selangor)	1	Study 5 vs Study 6	P = 0.486 df = 25 t = -0.707	P = 0.651 df = 25 t = 0.458
	2	Study 7 vs Study 8	P = 0.243 df = 22 t = -1.199	P = 0.443 df = 22 t = 0.781
Montane Forested Area (Bukit Cincin, Pahang)	1	Study 9 vs Study 10	P = 0.693 df = 60 t = 0.396	P = 0.950 df = 60 t = 0.063
	2	Study 11 vs Study 12	P = 0.702 df = 62 t = 0.384	P = 0.871 df = 62 t = 0.163

$p > 0.05$: not significantly different
$p \leq 0.05$: significantly different

Table 4.20. Comparison on mean ambient temperature, relative humidity and rainfall amount in different ecological habitats during the study periods.

Conditions	Ecological Habitats	Study Cases	One Way ANOVA		
			Temperature	Humidity	Rainfall
Outdoor	Lowland Forested Area (Ulu Gombak, Selangor)	Study 1, Study 3	P = 0.000 F = 105.26	P = 0.000 F = 10.37	P = 0.259 F = 1.34
	Coastal Area (Tanjong Sepat, Selangor)	Study 5, Study 7			
	Montane Forested Area (Bukit Cincin, Pahang)	Study 9, Study 11			
Indoor	Lowland Forested Area (Ulu Gombak, Selangor)	Study 2, Study 4	P = 0.000 F = 183.07	P = 0.000 F = 14.70	Not applicable because carcasses were not in contact with the rain water
	Coastal Area (Tanjong Sepat, Selangor)	Study 6, Study 8			
	Montane Forested Area (Bukit Cincin, Pahang)	Study 10, Study 12			

$p > 0.05$: not significantly different
$p \leq 0.05$: significantly different

The mean number of day on decomposition stages of monkey carcasses placed in outdoor and indoor located in different ecological habitats is shown in Figure 4.37. Generally, the duration of each decomposition stage on monkey carcasses placed in indoor was longer than outdoor in all ecological habitats. Decomposition duration on monkey carcasses placed indoor and outdoor in lowland (mean number of day: outdoor = 27 days, indoor = 63.5 days) and montane (outdoor = 43 days, indoor = 43 days) forested areas were longer than coastal area (outdoor = 9 days, indoor = 16.5 days).

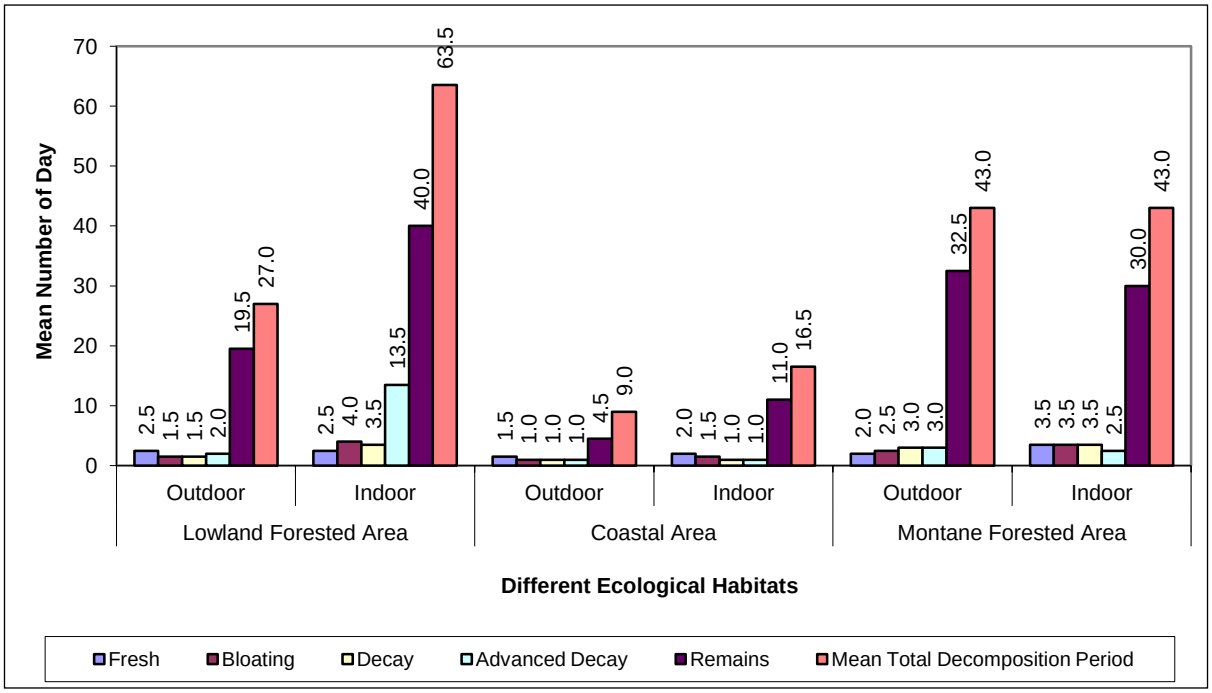

Figure 4.37. Mean number of day on decomposition stages of monkey carcasses placed in outdoor and indoor located in different ecological habitats.

Dipterans obtained from different decomposition stages of monkey carcasses in lowland forest, coastal and montane forest areas in Malaysia are shown in Figure 4.38, Figure 4.39 and Figure 4.40, respectively.

Figure 4.38 showed that adults of *Hypopygiopsis violacea*, *Chrysomya pinguis*, *Lucilia* spp. and *Scholastes* spp. only visited carcasses placed outdoor in lowland forested area in the early decomposition stages (fresh stage and bloating stage).

However, no larvae of *Lucilia* spp. and *Scholastes* spp. were found colonising the carcasses neither outdoor nor indoor, while *H. violacea* and *C. pinguis* were found colonising on the carcasses in both outdoor and indoor. On the other hand, larvae of *Chrysomya nigripes* and *Chrysomya rufifacies* only colonized on carcasses placed outdoor; and *Hemipyrelia* spp., *Musca domestica* and *Sarcophaga* spp. only colonized on carcasses placed indoor in lowland forested area in this particular study. *Chrysomya chani*, *C. megacephala*, *C. pinguis*, *C. villeneuve* and *Ophyra spinigera* were recorded as common colonizers on carcasses found in lowland forested area in both outdoor and indoor. *Ophyra spinigera* was observed to colonize on late decomposition stages (from decay stage until remains stage), while *Hermetia illucens* was found only on remains stage.

Figure 4.39 shows that adults of *Phumosia* spp., *Scholastes* spp. and Neriid were visiting carcasses placed outdoor in coastal area; however, none of these flies colonized any carcasses in this site. This was surprisingly and contrast with study conducted in lowland forested area, larvae of *Ophyra spinigera* and *Sarcophaga* spp. only colonized on carcasses placed outdoor and indoor, respectively. *Chrysomya megacephala*, *C. rufifacies* and *Hemipyrelia* were recorded as the common colonizer in coastal area for both outdoor and indoor.

Figure 4.40 shows that adults of *Stomorhina xanthogaster* and *Tabanus* spp. were collected from carcasses placed outdoor, and phorids were collected from carcasses placed indoor in montane forested area. However, none of these flies colonized the carcasses. Althought *C. megacephala* adults were observed to visit the carcasses in outdoor and indoor, *C. megacephala* larvae were only found on carcasses placed indoor. *Chrysomya pinguis*, *C. villeneuve*, *H. violacea*, *Lucilia porphyrina*, *Ophyra spinigera* and *Sarcophaga* spp. were recorded as common colonizers on carcasses placed in montane forested area. *Ophyra spinigera* were found only

colonizing on carcasses at late decomposition stages (from decay stage to remains stage) as reported in lowland forested area. In addition, flies belong to the family of Fanniidae, *Fannia canicularis* and *Fannia scalaris* were only found to colonise on carcasses at remains stage in both outdoor and indoor.

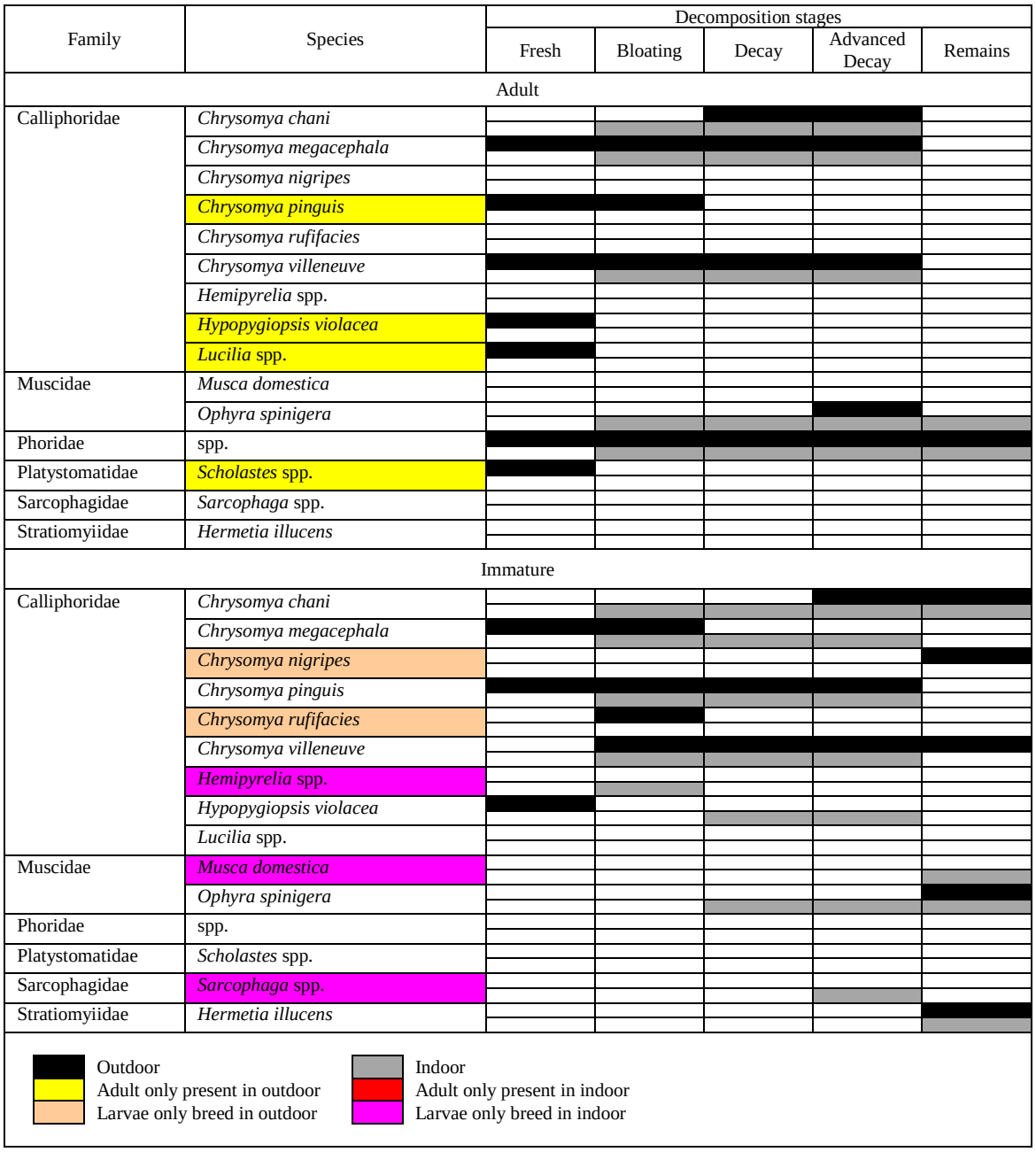

Figure 4.38. Dipterans obtained from different decomposition stages of monkey carcasses in lowland forested area in Malaysia.

Family	Species	Decomposition stages				
		Fresh	Bloating	Decay	Advanced Decay	Remains
Adult						
Calliphoridae	*Chrysomya megacephala*					
	Chrysomya rufifacies					
	Hemipyrelia spp.					
	Phumosia spp.					
Muscidae	*Musca domestica*					
	Ophyra spinigera					
Neriidae	spp.					
Phoridae	spp.					
Platystomatidae	*Scholastes* spp.					
Sarcophagidae	*Sarcophaga* spp.					
Immature						
Calliphoridae	*Chrysomya megacephala*					
	Chrysomya rufifacies					
	Hemipyrelia spp.					
	Phumosia spp.					
Muscidae	*Musca domestica*					
	Ophyra spinigera					
Neriidae	spp.					
Phoridae	spp.					
Platystomatidae	*Scholastes* spp.					
Sarcophagidae	*Sarcophaga* spp.					

Outdoor
Adult only present in outdoor
Larvae only breed in outdoor

Indoor
Adult only present in indoor
Larvae only breed in indoor

Figure 4.39. Dipterans obtained from different decomposition stages of monkey carcasses in coastal area in Malaysia.

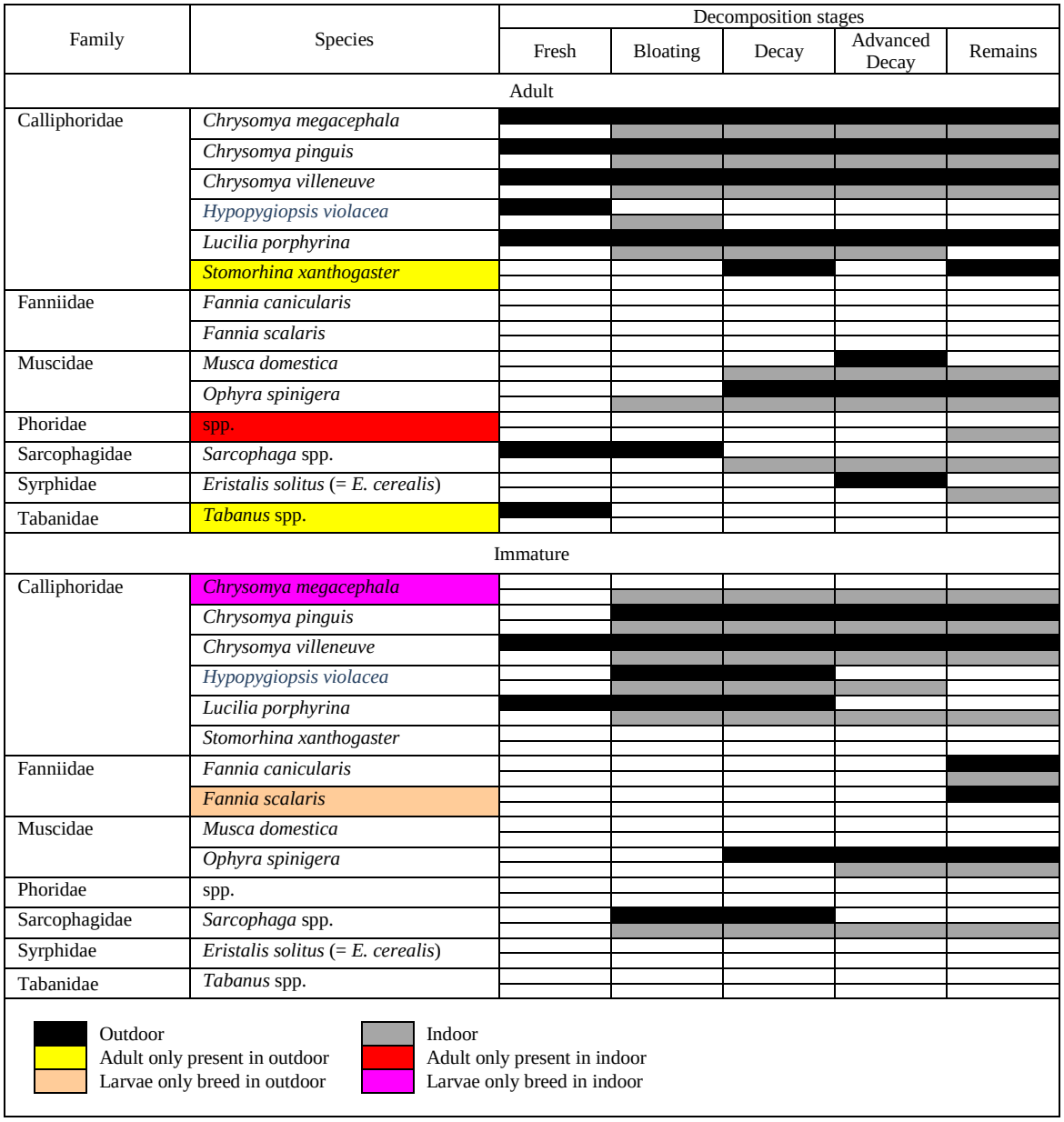

Figure 4.40. Dipterans obtained from different decomposition stages of monkey carcasses in montane forested area in Malaysia.

Table 4.21 summarises and compares between Diptera obtained from forensic entomological studies conducted in lowland forested area, coastal area and montane forested area in Malaysia. Generally, 23 species of flies belonging to 10 families were recorded in 12 forensic entomological studies conducted in three different ecological habitats. The presence of certain species of flies in different habitats may act as important forensic geographical indicator. Only dipterans ovipositing their offspring on the carcasses can be used for post mortem interval estimation and served as geographical indicators.

Chrysomya chani (adult and larvae), *Chrysomya nigripes* (larvae) and *Hermetia illucens* (larvae) were only found in lowland forested area; *Phumosia* spp. (adult) and Neriid (adult) were only found in coastal area; and *Lucilia porphyrina* (adult and larvae), *Stomorhina xanthogaster* (adult), *Fannia canicularis* (larvae), *Fannia scalaris* (larvae), *Eristalis solitus* (adult) and *Tabanus* spp. (adult) were only found in montane forested area. In addition, our study also recorded *Chrysomya pinguis*, *Chrysomya villeneuve* and *Hypopygiopsis violacea* only found colonizing on carcasses placed in forested area, including lowland and montane forest.

Chrysomya megacephala, *Ophyra spinigera* and *Sarcophaga* spp. were observed to be able to colonise on carcasses located in all ecological habitats. Thus, these three species are the most dominant species.

Table 4.21. Summary of diptera (adult and immature) obtained from 12 forensic entomological studies conducted in lowland forested area, coastal area and montane forested area in Malaysia.

Family	Species	Outdoor			Indoor			Remarks
		Lowland Forested Area	Coastal Area	Montane Forested Area	Lowland Forested Area	Coastal Area	Montane Forested Area	
Calliphoridae	*Chrysomya chani*	+			+			L
	Chrysomya megacephala	+	+	+	+	+	+	
	Chrysomya nigripes	+						L
	Chrysomya pinguis	+		+	+		+	F
	Chrysomya rufifacies	+	+			+		
	Chrysomya villeneuve	+		+	+		+	F
	Hemipyrelia spp.		+		+	+		
	Hypopygiopsis violacea	+		+	+		+	F
	Lucilia porphyrina			+			+	M
	Lucilia spp.	+						
	Phumosia spp.		+					C
	Stomorhina xanthogaster			+				M
Fanniidae	*Fannia canicularis*			+			+	M
	Fannia scalaris			+				M
Muscidae	*Musca domestica*		+	+	+	+	+	
	Ophyra spinigera	+	+	+	+	+	+	
Sarcophagidae	*Sarcophaga* spp.		+	+	+	+	+	
Stratiomyiidae	*Hermetia illucens*	+			+			L

Remarks:
L = Exclusive in lowland forested area
C = Exclusive in coastal area
M = Exclusive in montane forested area
F = Recorded in forested area only

4.7 FIRST REPORT ON THE SIGNAL FLY, *SCHOLASTES* SPP. (LOEW, 1873) (DIPTERA: PLATYSTOMATIDAE) ON ANIMAL CARCASSES IN MALAYSIA

After death was confirmed, monkey carcass was immediately placed ourdoor in a forest. Observers sat near the carcass to confirm the timing of the arrival of forensically important flies. Within an hour, the blowflies, *Hypopygiopsis* sp. (Townsend) were the first visitor to the carcass, followed by *Chrysomya* sp. (Robineau-Desvoidy). On the other hand, a signal fly, *Scholastes* sp. was also observed visiting the carcass. The *Scholastes* sp. was observed landing and sucking the blood stain on the cloth (Figure 4.41). No *Scholastes* sp. was observed visiting the monkey carcass after the first day. This study reported the occurrence of *Scholastes* sp. on animal carcasses in Malaysia for the first time.

Figure 4.41. *Scholastes* sp. was observed landing on the bloodstain on the cloth worn by a freshly dead monkey (*Macaca fascicularis*, Raffles).

4.8 FIRST REPORT OF THE HOUSE FLY LARVAE, *MUSCA DOMESTICA* (LINNAEUS) (DIPTERA: MUSCIDAE) ASSOCIATED WITH THE MONKEY CARCASS IN MALAYSIA

The species of maggots obtained from different decomposition stages of monkey carcass are showed in Figure 4.42. No fly and larva activities were observed on the carcass for the first 3 days of post mortem. On Day 4 (bloating), adults of *Chrysomya villeneuvi* Patton and *Chrysomya chani* Kurahashi (less than 10 flies) were observed to visiting the carcass, and egg masses were observed at the eye and mouth regions of the carcass. However, no larvae were found on the Day 4. On Day 5 (bloating) and Day 6 (bloating), second-instar larvae of *Chrysomya pinguis* Walker, *Ch. villeneuvi* and *Ch. chani* were collected from the carcass, indicating these three species of blow flies were the early colonizers on carcass placed indoor in forested area. Pupae of *Ch. villeneuvi* and *Ch. chani* were found on and around the carcass from the Day 10 (advanced decay) onwards.

The 3rd-instar larvae of *M. domestica* were only found on Day 33 (remains stage) of a decomposed monkey carcass. A total of 6 3rd-instar maggots of *M. domestica* were collected from the monkey carcass. No 1st-instar and 2nd-instar larvae and puparia of *M. domestica* were recovered from the carcass. *Musca domestica* maggots were recovered together with another muscid fly maggots, *Ophyra* (*=Hydrotaea*) *spinigera* Stein, on dry stage of a carcass. *Musca domestica* maggots were not obtained after this. *Ophyra spinigera* maggots were the dominant colonizer in the dry stage.

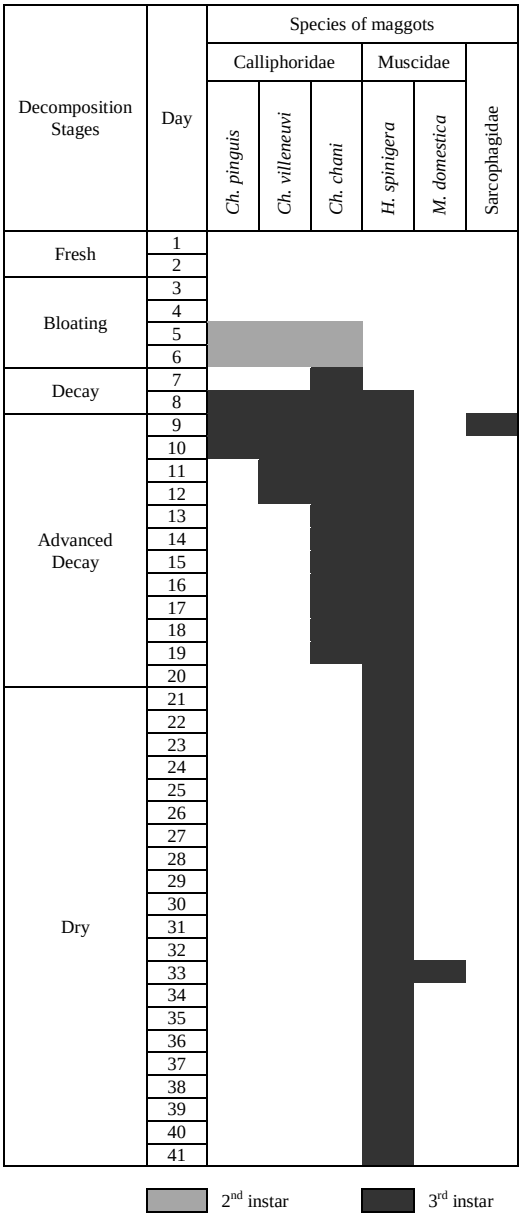

Figure 4.42. Maggots recovered from monkey carcass (*Macaca fascicularis* Raffles) placed in forested area in Ulu Gombak, Selangor from 9 May to 18 June 2007. One monkey carcass was used in this study.

4.9 ANTS (HYMENOPTERA: FORMICIDAE) RECOVERED FROM FORENSIC ENTOMOLOGICAL STUDIES CONDUCTED IN DIFFERENT ECOLOGICAL HABITATS IN MALAYSIA

Figure 4.43 shows ant species presence with relation to decomposition stage and ecological habitat. Each carcass passed through the five stages of decomposition: fresh, bloating, active decay, advanced decay and dry/remains. Within 15 minutes of death, ants were attracted to the blood of the freshly killed monkeys. Ants were observed in all the decomposition stages of the carcasses. These ants were observed to preying the eggs, maggots, pupae and newly emerged flies throughout the study period.

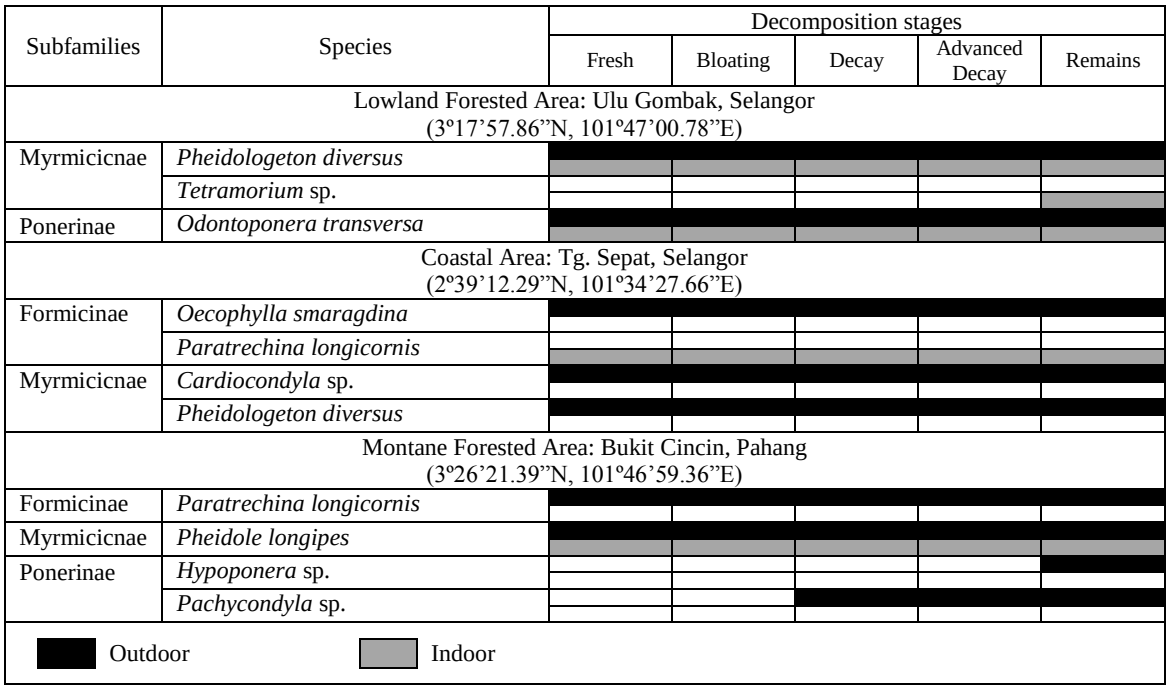

Figure 4.43. Species of ants obtained from different decomposition stages of monkey carcasses in lowland forested area, coastal area and montane forested area in Malaysia.

Table 4.22 presents a summary of ants recovered from different ecological habitats in forensic entomology studies. Only 3 species of ants belonging to 2 subfamilies were obtained from carcasses placed in lowland forested area, namely *Pheidologeton diversus* (Jerdon, 1851) (Formicidae: Myrmicicnae), *Tetramorium* sp. (Formicidae: Myrmicicnae) and *Odontoponera transversa* (Smith, 1857) (Formicidae: Ponerinae). On the other hand, 4 species of ants belonging to 2 and 3 subfamilies were found on carcasses placed in coastal area and montane forested area, respectively. The coastal area ant species were: *Oecophylla smaragdina* (Fabricius, 1775) (Formicidae: Formicinae), *Paratrechina longicornis* (Latreille, 1802) (Formicidae: Formicinae), *Cardiocondyla* sp. (Formicidae: Myrmicicnae) and *Pheidologeton diversus* (Formicidae: Myrmicicnae), while those found in the montane forested area were: *Paratrechina longicornis* (Formicidae: Formicinae), *Pheidole longipes* (Latreille, 1802) (Formicidae: Myrmicicnae), *Pachycondyla* sp. (Formicidae: Ponerinae) and *Hypoponera* sp. (Formicidae: Ponerinae).

Our results revealed that *Tetramorium* sp. was only found visiting carcasses placed indoors; while *Oecophylla smaragdina*, *Cardiocondyla* sp., *Hypoponera* sp. and *Pachycondyla* sp. were only found on carcasses placed outdoors.

In this study, ants were reported occurring in all the decomposition stages, indicating that ants were not a significant indicator for carcasses succession or post mortem interval estimation. However, we found that different species of ants visited the monkey carcasses placed in different ecological habitats (Table 4.22). *Cardiocondyla* sp. was only found on carcasses placed in the coastal area; while *Pheidole longipes*, *Hypoponera* sp. and *Pachycondyla* sp. were found on carcasses placed in the montane forested area.

We also noticed that some of the ants were found in more than one ecological habitat, e.g. *Paratrechina longicornis* was found in coastal and montane forested areas, and *Pheidologeton diversus* was found in coastal and lowland forested areas.

Table 4.22. Ants recovered from forensic entomological studies in forested area, coastal area, highland and oil palm plantation areas in Malaysia.

Subfamilies	Species	Study Site		
		Lowland Forested Area[a]	Coastal Area[a]	Montane Forested Area[a]
Formicinae	*Oecophylla smaragdina* (Fabricius)		+	
	Paratrechina longicornis (Latreilla)		+	+
Myrmicicnae	*Cardiocondyla* sp.		+	
	Pheidole longipes (Latreille)			+
	Pheidologeton diversus (Jerdon)	+	+	
	Tetramorium sp.	+		
Ponerinae	*Hypoponera* sp.			+
	Odontoponera transversa (Smith)	+		
	Pachycondyla sp.			+

[a]4 Replicates (2 monkey carrions placed indoor and 2 monkey carrions placed outdoor)
+Presence of ant

4.10 BEETLES (COLEOPTERA) RECOVERED FROM FORENSIC ENTOMOLOGICAL STUDIES CONDUCTED IN DIFFERENT ECOLOGICAL HABITATS IN MALAYSIA

A total of 24 Coleoptera species belonging to 12 families was recorded on monkey carcasses placed outdoor and indoor (Table 4.23). Figure 4.44 and Figure 4.45 show the patterns of Coleoptera succession in different decomposition stages on carcasses placed outdoor and indoor in lowland and montane forested areas. There was no beetle found in forensic entomological studies conducted in coastal area.

In lowland forested area, a total of 13 species of beetles belonging to 8 families were collected from forensic entomological studies conducted in outdoor and indoor (Figure 4.44). Beetles obtained from monkey carcasses placed indoor (11 species) were more diverse than outdoor (5 species). Generally, beetles were found from all decomposition stages on carcasses placed in a lowland forested area. *Phaeochroops rattus* (Coleoptera: Hybosoridae) was collected as early as in fresh stage. All collected beetles specimens in a lowland forested area were adults, except larvae of *Chrysosilpha formosa* (Coleoptera: Silphidae) that were collected from carcasses placed indoor in advanced decay and remains stages.

In a montane forested area, a total of 15 species of beetles belonging to 8 families were collected from forensic entomological studies conducted in outdoor and indoor (Figure 4.45). Beetles were only observed to visit the carcasses from bloating stage onwards in montane area. The diversity of beetles obtained from carcasses placed outdoor (9 species) and indoor (10 species) was not much different. The species of beetles collected from carcasses increased from bloating stage to remains stage in a study conducted in both outdoor and indoor (Figure 4.46) as observed in a lowland forested area. Majority of beetle specimens obtained from studies conducted in montane

forested area were adults, only larvae of *Chrysosilpha formosa* (Coleoptera: Silphidae) and *Philonthus* sp. (Coleoptera: Staphylinidae) were collected from carcasses placed outdoor on remains stage.

The species of beetles collected from carcasses increased from fresh stage to remains stage in studies conducted in outdoor and indoor in both study sites (Figure 4.46), indicating that beetles preferred to colonize late decomposition stages of monkey carcasses.

Beetles that visited carcasses placed in montaned forested area (15 species) were more diverse compared to a lowland forested area (13 species) (Table 4.23). Staphylinids were dominant on carcasses placed in a montane forested area; while Hybosorids were dominant on carcasses placed in a lowland forested area. Only 4 species [*Sphaeridium* spp. (Hydrophilidae), *Chrysosilpha formosa* (Silphidae), *Aleochara* spp. (Staphylinidae) and *Platydracus* (Staphylinidae)] beetles were found to visit carcasses placed in both ecological habitats, while the rest was geographically specific. This indicates that beetles not only can be used for PMI estimation, but also as a geographical indicator.

Family	Species	Decomposition Stages				
		Fresh	Bloating	Decay	Advance Decay	Remains
Hybosoridae	*Phaeochroops rattus*					
	Phaeochroops peninsularis					
	Phaeochrous emarginatus					
Hydrophilidae	*Sphaeridium* spp.					
Lampyridae	Gen. spp.					
Lycidae	Gen. spp.					
Scarabaeidae	*Onthophagus* nr. *babirussa*					
	Onthophagus rudis					
Silphidae	*Chrysosilpha formosa*				L	L
Staphylinidae	*Aleochara* spp.					
	Platydracus spp.					
	Gen. spp.					
Tenebrionidae	Gen. spp.					

Outdoor Indoor L Larvae

Figure 4.44. Species of beetles (Coleoptera) obtained from different decomposition stages of monkey carcasses in a lowland forested area in Malaysia.

Family	Species	Decomposition Stages				
		Fresh	Bloating	Decay	Advance Decay	Remains
Chrysomelidae	Gen. sp.					
Curculionidae	Gen. sp.					
Elateridae	Gen. sp.					
Hydrophilidae	*Cercyon* sp.					
	Sphaeridium sp.					
Leiodidae	*Nemadus* sp.					
Scarabaeidae	*Sericania* sp.					
Silphidae	*Chrysosilpha formosa*					L
Staphylinidae	*Aleochara* sp.					
	Atheta sp.					
	Creochara brevipennis					
	Hesperus sp.					
	Ontholestes sp.					
	Philonthus sp.					L
	Platydracus sp.					

Outdoor Indoor L Larvae

Figure 4.45. Species of beetles (Coleoptera) obtained from different decomposition stages of monkey carcasses in montane forested area in Malaysia.

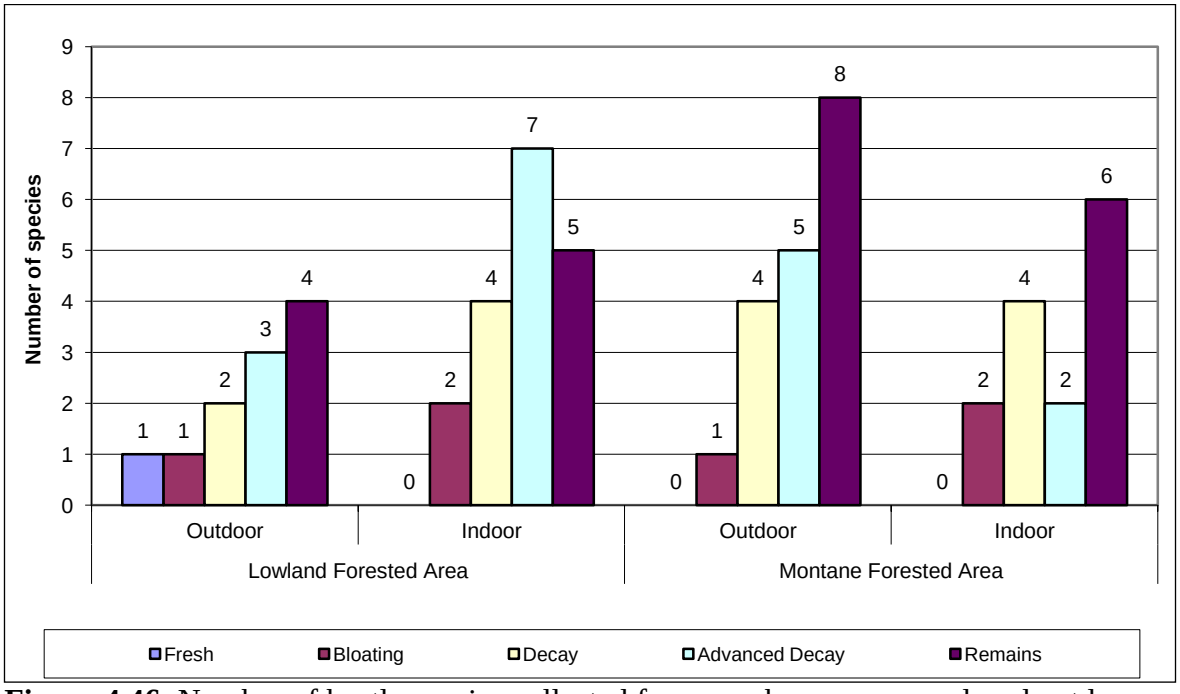

Figure 4.46. Number of beetle species collected from monkey carcasses placed outdoor and indoor in lowland and montane forested areas in Malaysia. Generally, number of beetle species obtained from fresh stage to remains stage increased.

Table 4.23. Summary of beetles recovered from forensic entomological studies in lowland and montane forested areas in Malaysia.

Family	Species	Study Sites	
		Lowland Forest	Montane Forest
Chrysomelidae	unidentified sp.		+
Curculionidae	unidentified sp.		+
Elateridae	unidentified sp.		+
Hybosoridae	*Phaeochroops rattus* (Arrow, 1909)	+	
	Phaeochroops peninsularis (Arrow, 1909)	+	
	Phaeochrous emarginatus (Laporte, 1840)	+	
Hydrophilidae	*Cercyon* sp. (Leach, 1817)		+
	Sphaeridium sp. (Fabricius, 1775)	+	+
Lampyridae	unidentified sp.	+	
Leiodidae	*Nemadus* sp. (Thomson, 1867)		+
Lycidae	unidentified sp.	+	
Scarabaeidae	*Onthophagus* nr. *babirussa* (Eschscholtz, 1822)	+	
	Onthophagus rudis (Sharp, 1875)	+	
	Sericania sp. (Motschulsky, 1860)		+
Silphidae	*Chrysosilpha formosa* (Laporte, 1928)	+	+
Staphylinidae	*Aleochara* sp. (Gravenhorst, 1802)	+	+
	Atheta sp. (Thomson, 1858)		+
	Creochara brevipennis (Cameron, 1939)		+
	Hesperus sp. (Fauvel, 1874)		+
	Ontholestes sp. (Ganglbauer, 1895)		+
	Philonthus sp. (Curtis, 1829)		+
	Platydracus sp. (Thomson, 1858)	+	+
	unidentified sp.	+	
Tenebrionidae	unidentified sp.	+	

+ Presence of beetles

4.11 FAUNA SUCCESSION AND DECOMPOSITION ON INCINERATED MONKEY CARCASSES

Our study was extended to observe the incineration effect on fauna succession and decomposition of monkey carcasses. A total of 2 replicates were conducted concurrently with Study 1 (Outdoor 1, Diurnal) and Study 3 (Outdoor 2, Nocturnal) at the same study site, respectively. Hence, this study had similar ambient temperature, relative humidity and daily rainfall amount as reported in Study 1 (Section 4.1.1) and Study 3 (Section 4.1.3).

After the monkeys were euthanised, the carcasses were incinerated by diesel. In this study, the carcasses were incinerated to give a Crow-Glassman Scale (CGS) (Table 4.24) at level 2 with 100% body surface / skin burned. The incineration of monkey carcasses took about 20 minutes and the cooling process took about 15 minutes. The carcasses were considered cool when no smoke was observed and no heat was felt when probed with a hand placed near the carcasses.

Table 4.24. Description of Crow-Glassman Scale (CGS) in different level of burning effect (Glassman & Crow, 1996). The CGS of thermal alteration was divided into five levels.

Level	Terms	Description
1	Recognizable for identification	• Typical of smoke death, with possible epidermal blistering and singeing of the hair.
2	Possibly recognizable	• Varying degrees of charring on elements such as the hands/feet, genitalia and ears.
3	Non recognizable	• Major destruction/disarticulation of the head and extremities.
4	Extensive burn destruction	• The skull and extremities are severely fragmented or missing.
5	Cremation	• Little or no tissue remains and osteological fragments are scattered and incomplete.

The decomposition of carcass in this study had undergone 4 stages namely, fresh (2 days), decay (1 – 2 days) advanced decay (1 day) and remains (11 – 20 days). Due to the incineration effect, the guts from the abdominal cavity were exposed, thus no bloating stage was observed. Summary of insect succession on incinerated monkey carcasses was shown in Table 4.25.

Table 4.25. Summary of insect succession on incinerated monkey carcasses in Ulu Gombak, Selangor, Malaysia.

Decomposition Stages		Fresh	Decay	Advanced Decay	Remains
Duration of Decomposition Stages (Day)		2	1 – 2	1	11 – 20
Mean Duration of Decomposition Stages (Day)		2	1.5	1	15.5
Calliphoridae	*Chrysomya chani*	A	A	A	A, L, P, NE
	Chrysomya megachephala	#1, A	A, L	A	
	Chrysomya pinguis	A	A	A, L	L
	Chrysomya villeneuve	A	A	A	L, P, NE
	Hypopygiopsis violacea	#1, A	L	L	L
Muscidae	*Ophyra spinigera*		A	A	A, L
Sarcophagidae	spp.	A			
Formicidae	*Pheidologeton* spp.	A	A	A	A
Histeridae	*Margarinotus* spp.				A
Scarabaeidae	*Onthophagus egregius*		A		A
Silphidae	*Chrysosilpha formosa*				A
Staphylinidae	*Hesperus* spp.				A
	Platydracus spp.				A

#1 = First fly visit to the carcass; A = Adult; L = Larva; P = Pupae; NE = Newly emerged adult

Fresh stage (Duration = 2 Days). After the incineration, the cloth and fur on the carcasses were totally burned away. The body surface was completely charred, skin was chapped and muscles were exposed and ruptured (Figure 4.47a). In addition, the abdominal cavity was exposed and the guts were burned and blackened (Figure 4.47b). No liquefacation of the carcasses was observed.

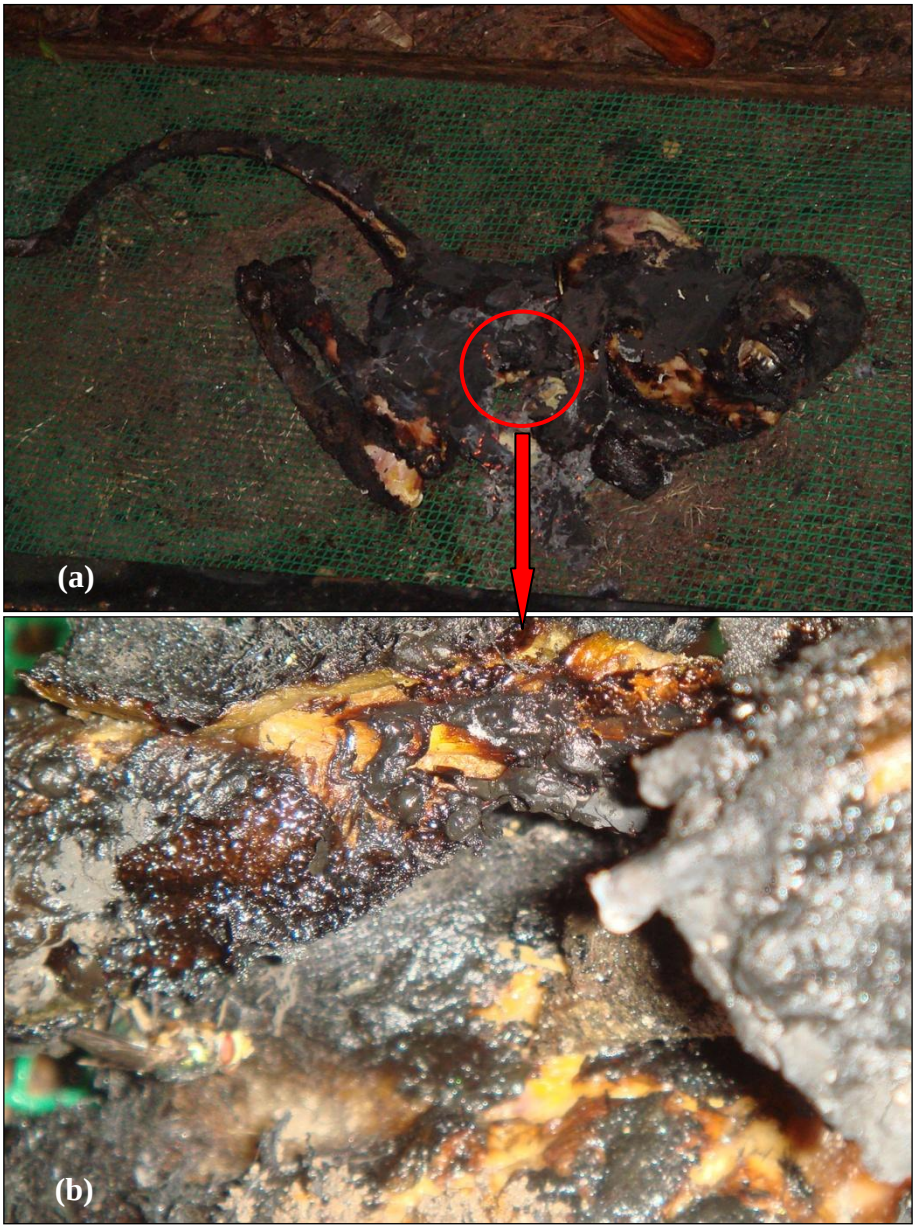

Figure 4.47. (a) Incinerated monkey carcass; and (b) the incineration effect which exposed the guts, thus no bloating stage was observed.

On Day 1 (fresh stage), adult blowflies, *Chrysomya megacephala* (Fabricius) and *Hypopygiopsis violacea* (Macquart) were seen resting on the exposed guts and stomach contents about 30 minutes after the carcass was incinerated. Ants, *Pheidologeton* sp. (Hymenoptera: Formicidae) were seen visiting the carcass within 1 hour after incineration. First batch of fly eggs was seen on the stomach region, followed by mouth region. Ants (*Pheidologeton* sp., Myar) were found attracted to the fluid from the guts and predating on the eggs.

On Day 2 (fresh stage), a lot of ants were observed preying on the fly eggs and making their nest around the chest, stomach and anus regions of the monkey carcass (Figure 4.48a). Many calliphorines [*Ch. megacephala*, *Chrysomya villeneuve* (Patton), *Chrysomya chani* (Kurahashi) and *Chrysomya pinguis* (Walker)] sarcophagids were seen around and landing on the carcass. The fresh stage lasted for 2 days.

Figure 4.48. The ants of *Pheidologeton* sp. (Hymenoptera: Formicidae) were making their nest on the carcass from Day-2 (fresh) until Day 5 (advanced decay).

Decay stage (Duration = 1 – 2 Days, Mean = 1.5 Days). Decay stage began when the maggot mass was observed actively consuming the soft tissues of the stomach, anus, neck and head regions of the monkey carcasses. More flies were attracted to the carcasses due to strong odour that was emanated from the carcasses. The carcasses were dominated by calliphorine larvae.

On Day-3 (decay stage), maggot masses were observed at the stomach, anus, neck, mouth and head regions of the monkey carcasses. The maggots were identified as 2nd- and 3rd-instar maggots of *H. violacea* and *C. megacephala*. The ants (*Pheidologeton* sp.) and scarab beetles (*Onthophagus* sp.) were found predating on the maggots. Adult calliphorines and muscids were found visiting the carcass. *Chrysomya megacephala* and *Ch. villeneuve* were dominant adult flies collected around the carcass, followed by *Ch. chani*, *Ch. pinguis* and *Ophyra spinigera* (Stein).

Advanced decay stage (Duration = 1 Day). Percentage of remaining biomass decreased rapidly, and majority of bones and cartilages was exposed in advanced decay stage. The internal organs and tissues under the skin were completely consumed by the maggots. Maggot mass could be seen around and under the carcass. The odour begun to reduce. Less adult calliphorines were seen, in comparison to decay stage. No liquefication was observed at the end of this stage. Third-instar maggots of *Ch. pinguis*, *Ch. villeneuve* and *Ch. chani* were collected on advanced decay stage.

Remains stage (Duration = 11 – 20 Days, Mean = 15.5 Days). The remains of the carcasses were bones, cartilages and skin layers. Percentage of remaining biomass decreased slowly until it stabilised. Much lesser or no odour was noticed in this stage. On Day 6 (remains stage), maggot mass can be seen around and under the carcass. Some maggots burrowed under the soil preparing for pupation. *Ch. villeneuve and Ch. chani* were dominant maggots mass in early remains stage (Day 6 – Day 9). Pupae of *C. chani* and *C. villeneuve* were found under the carcasses on Day 7 and Day 10,

respectively. Ants were seen predating the pupae actively. On Day 7, scarab beetles (*Onthophagus* sp.) and rove beetles (*Platydracus* sp.) were found on the carcass.

Second and third-instar maggots of *O. spinigera* were found colonising the skeletonized carcasses from Day 8 onwards. Acalypterate flies belonging to the families Drosophilidae, Dolicopodidae, Lauxaniidae and Platystomatidae (*Euthyplatystoma* sp.) were found attracted to the skeletons. Very minimum calliphorines were observed in this stage. Five species of beetles belonging to 4 families were obtained from the skeletonised carcass, namely Silphidae (*Chrysosilpha formosa*), Scarabaeidae (*Onthophagus* sp.), Staphylinidae (*Platydracus* sp. and *Hesperus* sp.) and Histeridae (*Margarinotus* sp.).

On Day 10, 11, 14 and 15, newly emerged *C. villeneuve* and *C. chani* were noticed landing onto the skeletons, fence and shrubs near by the carcasses. Maggots of *O. spinigera* were found burrowing under the soil from Day 12 onwards. Only ants and beetles were found on the remains at the end of this study.

The comparison between insect fauna obtained from incinerated and non-incinerated carcasses is shown in Table 4.26. Generally, fly and ant species obtained from non-incinerated carcasses were slightly more diverse than incinerated carcasses and vice versa for beetles.

The comparison on the duration of decomposition stages between incinerated and non-incinerated monkey carcasses is shown in Table 4.27. Incinerated carcasses required 4 – 5 days to fully decompose to remains or skeletonized; while non-incinerated carcasses required 7 – 8 days to become remains, indicating that the decomposition rate of incinerated carcasses was faster compared to non-incinerated carcasses.

This study emphasizes that (1) incineration effect does not deter the arrival and oviposition of forensically important flies on the carcasses; (2) incineration effect does

not make any difference on the fauna succession pattern between incinerated and non-incinerated carcasses; and (3) decomposition rates on incinerated carcasses are faster than non-incinerated carcasses.

Table 4.26. Comparison on the forensically important insect obtained from incinerated and non-incinerated monkey carcasses in a lowland forested area, Ulu Gombak, Selangor, Malaysia. Only flies (Calliphoridae, Muscidae and Sarcophagidae), ants and beetles are shown in the list.

List of forensically important insects			Presence of forensically important insects	
Order	Family	Species	Incinerated carcasses*	Non-incinerated carcasses*[#]
Diptera	Calliphoridae	*Chrysomya chani*	+	+
		Chrysomya megachephala	+	+
		Chrysomya pinguis	+	+
		Chrysomya rufifacies		+
		Chrysomya villeneuve	+	+
		Hypopygiopsis violacea	+	+
	Muscidae	*Ophyra spinigera*	+	+
	Sarcophagidae	unidentified sp.	+	+
	Stratiomyiidae	*Hermetia illucens*		+
Hymenoptera	Formicidae	*Pheidologeton* sp.	+	+
		Odontoponera sp.		+
Coleoptera	Histeridae	*Margarinotus* sp.	+	
	Hybosoridae	*Phaeochroops rattus*		+
	Scarabaeidae	*Onthophagus egregius*	+	
		Onthophagus nr. *babirussa*		+
		Onthophagus rudis		+
	Silphidae	*Chrysosilpha formosa*	+	+
	Staphylinidae	*Hesperus* sp.	+	
		Platydracus sp.	+	

*2 replicates for each study
[#]Study 1 (Section 4.1.1) and Study 2 (Section 4.1.4)

Table 4.27. Comparison on the duration of decomposition stages between incinerated and non-incinerated monkey carcasses placed outdoor in lowland forested area.

Decomposition Stage	Duration of Decomposition stage			
	Incinerated carcasses*		Non-incinerated carcasses*[#]	
	Duration	Mean Duration	Duration	Mean Duration
Fresh	2	2	2 – 3	2.5
Bloating	Not occurred due to incineration effect which exposed the guts.		1 – 2	1.5
Decay	1 – 2	1.5	1 – 2	1.5
Advanced Decay	1	1	2	2
Remains	11 – 20	15.5	8 – 31	27.0
Total Duration of Decomposition	16 – 24	20	15 – 39	34.5
Duration of carcasses fully decomposed to remains stage	4 – 5	4.5	7 – 8	7.5

*2 replicates for each study
[#]Study 1 (Section 4.1.1) and Study 2 (Section 4.1.4)

4.12 FAUNA SUCCESSION AND DECOMPOSITION ON MONKEY CARCASSES SUBMERGED IN FRESH WATER RIVER

Our study preceded to investigate the fauna succession and decomposition on monkey carcasses submerged in fresh water river. A total of 2 replicates were conducted concurrently with Study 1 (Outdoor 1, Diurnal) and Study 3 (Outdoor 2, Nocturnal) at the same study site, respectively. The river was about 20 meters away from the outdoor study site. Hence, this study had similar ambient temperature, relative humidity and daily rainfall amount as reported in Study 1 (Section 4.1.1) and Study 3 (Section 4.1.3).

After the monkeys were euthanised, the carcasses were placed in a metal cage and submerged into the river (Figure 4.49). River water temperatures were from 20.0 to 22.0ºC.

The carcasses had undergone 6 decomposition stages and were defined according to Byrd & Castner (2001), namely submerged fresh (2 – 3 days), early floating (3 – 4 days), floating decay (1 – 2 days), bloated deterioration (2 – 3 day), floated remains (2 days) and sunken remains (not determined). Sequence of insect succession on monkey carcasses submerged in fresh river water are summarised in Table 4.28.

Table 4.28. Summary of insect succession on monkey carcasses submerged in freshwater river in Gombak, Selangor, Malaysia.

Decomposition Stages		Submerged Fresh	Early Floating	Floating Decay	Bloated Deterioration	Floating Remains	Sunken Remains
Duration of Decomposition Stages (Day)		2 – 3	3 – 4	1 – 2	2 – 3	2	Not Determined
Mean Duration of Decomposition Stages (Day)		2.5	3.5	1.5	2.5	2	Not Determined
Calliphoridae	*Chrysomya megachephala*		A	A	A		
	Chrysomya pinguis			L	L	L	
	Hemipyrelia sp.		L	L	L		
Muscidae	*Ophyra chalcogaster*				A		
	Ophyra spinigera		A	A	A	A, L	
Sarcophagidae	Unidentified sp.			L	L	L	
Dolichopodidae	Unidentified sp.	A	A	A			
Lauxaniidae	Unidentified sp.	A	A	A			

A = Adult; L = Larvae

Figure 4.49. (a) The environment of study site. The metal cage was tighted with a rope to avoid being flushed away by the river water. (b) The monkey carcass was kept in a metal cage and right lateral aspect of the carcass was exposed from the water surface. The submersion condition on the carcass was depended on the daily water lever of the river.

Submerged fresh stage (Duration = 2 – 3 Days, Mean = 2.5 Days). Both carcasses did not fully submerge when placed in the river. Blood secreted from the gunshot wound was flush away by the water. The lateral aspect of the carcasses was exposed to the water surface. The submersion condition of the carcasses depended on the daily water lever of the river. The water lever in the river was fluctuated and

influenced by the climate, especially rainfall. The monkey carcasses remained unchanged for the first 2 – 3 days. No odour was noticed in this stage. On Day 1 (fresh), no insect activities were observed on the monkey. On Day 2 (fresh), adult flies belonging to the families Dolichopodidae and Lauxaniidae were observed visit the carcasses and no forensically important flies visited carcasses in Study 1 until Day 3.

Early floating stage (Duration = 3 – 4 Days, Mean = 3.5 Days). This stage was noticed when the abdomen of the carcasses started to swell. This was due to the anaerobic bacteria respiration in the abdomen that produced gases to push the abdomen above the water surface. The abdomen appeared greenish discoloration. The skin color became pale. Faint odour was noticed in this stage. Adult of *Ch. megacephala* and *O. spinigera* began to visit the monkey carcasses in early bloating stage on Day 4 and Day 5, respectively.

Floating decay stage (Duration = 1 – 2 Days, Mean = 1.5 Days). Floating decay stage began when the maggots mass started to concentrate on the monkey carcasses. The hair and skin began to slough off. Strong odour emanated from the carcasses. Maggot mass of *Ch. pinguis* (2nd-instar) were first obtained from tight region of the carcasses on Day 6, and *Hemipyrelia* sp. (3rd-instar) were first found in the opening on abdomen region (covered by cloth) of the carcasses on Day 7. Both species of maggots were present until Day 11 (floating remains). In addition, 3rd-instar maggots of Sarcophagidae were found on Day 8 (floating decay) until Day 13 (floating remains). *Chrysomya megacephala* (15 – 20 flies/collection/day) were dominant adult flies collected around the carcass in floating decay stage (Day 6 – Day 8), followed *O. spinigera* (<10 flies/collection/day). However, none of these species of maggots was obtained in this stage.

Bloated deterioration stage (Duration = 2 – 3 Days, Mean = 2.5 Days). During this stage, most of the tissues were consumed by maggots. Bones and cartilages were

exposed and the internal organs of the carcasses were observed. The hair was totally sloughed off. The maggots were seen migrating from the carcasses for pupation. However, many maggots were forced into water and flushed away by the water. The odour begun to fade away. *Ophyra spinigera* (5 – 9 flies/collection/day) became dominant adult flies in bloated deterioration stage (Day 8 – Day 10), compared to *Ch. megacephala* (2 – 3 flies/collection/day). Some maggots were observed migrating from the carcasses and crawling onto the metal cages on Day 10 (bloated deterioration). However, many of them were found dead near the carcasses and debris near by.

Floating remains stage (Duration = 2 Days, Mean = 2 Days). Very few maggots remained on the carcasses exposed above the water surface in this stage. Dead maggots were observed near the carcasses and debris around the metal cage. Hair was flushed away by the water. The organs and bones were observed projecting towards the water surface. The organs seemed to be clean and clear, which might be due to the fluid and dirt being washed way by the running freshwater. Partially remains stage sunk into the water. Less odor was noticed. This stage lasted until all remains were totally sunk into the water. *Ophyra spinigera* (3rd-instar) maggots were only found on 1 carcass on Day 11, because the carcass remain sunk into the water on the following day. No pupae of any fly species were obtained from both carcasses in this study.

Sunken remains stage (Duration = Not determined). The remains of the carcasses (bones, cartilages and some skin layers) sunk into water and remained in the bottom of the metal cage (below water surface). The study ended once the decomposition process entered this stage. No further observation was conducted on this stage after this.

In the first replicate, 2nd-instar maggots of *Ch. pinguis* were obtained on Day 6. The development of calliphorid maggots from 1st-instar to 2nd-instar took a minimum

time of 48 hours (2 days) and the estimated period for eggs hatching after oviposition was about 24 hours (1 day). Thus, the estimated minimum PMI was 3 days.

In the second replicate, 3rd-instar maggots of *Hemipyrelia* spp. were obtained on Day 7. The development of calliphorid maggots from 1st-instar to 3rd-instar took a minimum time of 72 hours (3 days) and the estimated period for eggs hatching after oviposition was about 24 hours (1 day). Thus, the estimated minimum PMI was 4 days.

Data from both studies estimated that the monkeys were killed on Day 3 assuming the flies were oviposited the eggs immediately on Day 3. Thus PMI estimation on carcasses submerged in freshwater river was delayed by a minimum period of 2 days.

Comparison between the forensically important insects obtained from monkey carcasses submerged in freshwater river and monkey carcasses placed outdoor in lowland forested area is shown in Table 4.29. Our results revealed that the flies that visited monkey carcasses placed outdoor were more diverse than monkey carcasses submerged in freshwater river. No ants and beetles were collected from monkey carcasses submerged in freshwater river.

Generally, carcasses submerged in freshwater river took about 9 – 11 days to reach floating remains stage, in comparison to carcasses placed outdoor on ground only took about 7 – 8 days to reach remains stage (Table 4.30). The results indicated that the decomposition rate of carcasses submerged in freshwater was slower than carcasses placed outdoor by 2 – 3 days.

This study emphasize that (1) PMI estimation on carcasses submerged in freshwater river was delayed by a minimum period of 2 days; (2) there was no difference on the fauna succession pattern between carcasses submerged in freshwater river and placed outdoor, with the sequence from Calliphoridae, Sarcophagidae and Muscidae; (3) *Ch. megacephala* was the dominant adult flies observed visiting the

carcasses, but only *Ch. pinguis* and *Hemipyrelia* sp. were the dominant maggots colonizing the carcasses; and (4) decomposition rates on carcasses submerged in freshwater river were slower than carcasses placed outdoor.

Table 4.29. Comparison between the forensically important insects obtained from monkey carcasses submerged in freshwater river and monkey carcasses placed outdoor in lowland forested area in Gombak, Selangor, Malaysia. Only flies (Calliphoridae, Muscidae and Sarcophagidae), ants and beetles were shown in the list.

List of forensically important insect			Present of forensically important insect	
Order	Family	Species	Carcasses submerged in freshwater river*	Carcasses placed outdoor*[#]
Diptera	Calliphoridae	*Chrysomya chani* (Kurahashi)		+
		Chrysomya megachephala (Fabricius)	+	+
		Chrysomya pinguis (Walker)	+	+
		Chrysomya rufifacies (Macquart)		+
		Chrysomya villeneuve (Patton)		+
		Hemipyrelia sp. (Townsend)	+	
		Hypopygiopsis violacea (Macquart)		+
	Muscidae	*Ophyra chalcogaster* (Wiedemann)	+	
		Ophyra spinigera (Stein)	+	+
	Sarcophagidae	unidentified sp.	+	+
	Stratiomyiidae	*Hermetia illucens* (Linnaeus)		+
Hymenoptera	Formicidae	*Pheidologeton* sp. (Mayr)		+
		Odontoponera sp. (Mayr)		+
Coleoptera	Hybosoridae	*Phaeochroops rattus* (Arrow)		+
	Scarabaeidae	*Onthophagus* nr. *Babirussa* (Eschscholtz)		+
		Onthophagus rudis (Sharp)		+
	Silphidae	*Chrysosilpha formosa* (Portevin)		+

*2 replicates for each study
[#]Study 1 (Section 4.1.1) and Study 2 (Section 4.1.4)

Table 4.30. Comparison on the duration of decomposition stages between carcasses submerged in freshwater river and placed outdoor in Ulu Gombak, Selangor, Malaysia.

Carcasses submerged in freshwater river*			Carcasses placed outdoor*[#]		
Decomposition Stages	Duration of Decomposition Stages (Day)	Mean Duration of Decomposition Stages (Day)	Decomposition Stages	Duration of Decomposition Stages (Day)	Mean Duration of Decomposition Stages (Day)
Submerged Fresh	2 – 3	2.5	Fresh	2 – 3	2.5
Early Floating	3 – 4	3.5	Bloating	1 – 2	1.5
Floating Decay	1 – 2	1.5	Decay	1 – 2	1.5
Decay Deterioration	2 – 3	2.5	Advanced Decay	2	2
Floating Remains	2	2	Remains	8 – 31	19.5
Sunken Remains	Not determined				
Duration took before reached floating remains stage	9 – 11	10	Duration took before reached remains stage	7 – 8	7.5

*2 replicates for each study
[#]Study 1 (Section 4.1.1) and Study 2 (Section 4.1.4)

4.13 FAUNA SUCCESSION AND DECOMPOSITION ON MONKEY CARCASSES TREATED WITH INSECTICIDE

Our study was extended to study the fauna succession and decomposition on monkey carcasses treated with an insecticide, malathion. A total of 2 replicates were conducted concurrently with Study 1 (Outdoor 1, Diurnal) and Study 3 (Outdoor 2, Nocturnal) at the same study site, respectively. The monkey carcasses placed outdoor was about 30 meters apart from the monkey carcasses trested with malathion. Hence, this study had similar ambient temperature, relative humidity and daily rainfall amount as reported in Study 1 (Section 4.1.1) and Study 3 (Section 4.1.3).

Generally, the decomposition of carcasses had undergone 5 stages namely, fresh (2 days), bloating (5 days), decay (6 days) advanced decay (2 days) and remains (4 days). Summary of insect succession on monkey carcasses treated with malathion was shown in Figure 4.50.

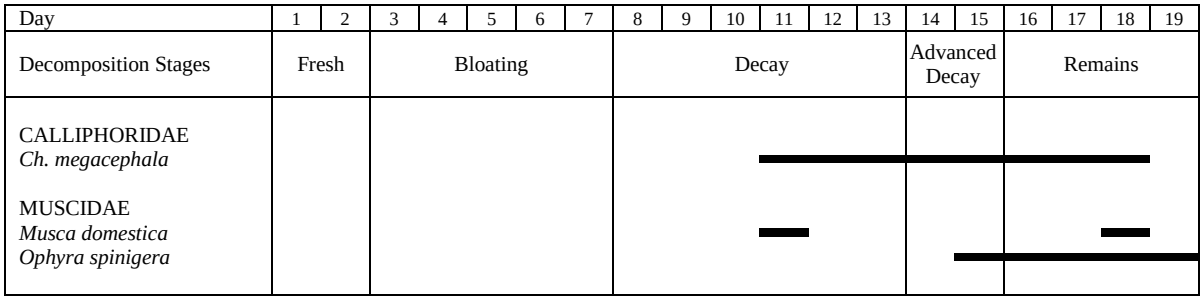

Figure 4.50. Fly maggots obtained from monkey carcasses treated with malathion in 5 decomposition stages in a forested area. Bars (black) indicate presence of fly maggots.

Fresh stage (Duration = 2 Days). On Day 1, malathion applied on the carcasses was absorbed by the cloth. The odour of the insecticide was strong and pungent. Adult flies of *Ch. megacephala* and *Ch. villeneuve* were observed landing on the fence around the carcasses 30 minutes after the carcasses were treated with malathion. However, no

adult flies were observed landing on the carcasses treated with malathion. There was no oviposition on the first day. No ants were observed visiting the carcasses.

On Day 2, the carcasses were hardened and the carcass stomach was slightly bloated. No liquefacation of the carcasses was observed. A small amount of fly eggs (about 1cm of diameter) were observed on the neck region of the carcasses but none of them hatched. Flies that landed on the carcasses were killed by the insecticide.

Bloating stage (Duration = 5 Days). The odour of the insecticide was still strong and pungent. The carcasses bloated since Day 2 till Day 4 with optimum remaining biomass at 114.79%. The percentage of remaining biomass then decreased from Day 4 (114.79%) till Day 7 (100.21%). The bloating stage ended when the abdomen of the carcasses shrunken. The body hairs were lost in this period. No liquefacation of the carcasses were observed.

From Day-3 (bloating stage) till Day-10 (decay stage), dead flies were found around the carcasses and no maggots were recovered. Dead dipterans obtained on and around the carcasses were those belonging to the families of Calliphoridae (*Ch. megacephala*, *Ch. rufifacies*, *Ch. velleneuve*, *Ch. nigripes* and *Ch. chani*), Muscidae (*O. spinigera*, *O. chalcogaster*, *M. domestica*), Lauxaniidae, Platystomatidae (*Euthyplatystoma* spp.), Dolichopodidae and Sarcophagidae (Table 4.31). Other dead fauna obtained on and around the carcasses were beetles, ants, cockroaches, ticks and butterfly (Table 4.31).

Decay stage (Duration = 6 Days). The decay stage was noticed when the abdomen of the carcasses begun to shrunk and flatten. The odour of insecticide was wanning from Day 8 to Day 13. However, a strong odour of fermented material was noticed. On Day 11, the guts of the carcasses were exposed and a yellowish viscous medium resulting from liquefication of fats degradation was observed. The leg regions were decaying and bones of the legs were observed. Maggots were observed on the

head, mouth and anus region of the carcasses. Percentage of remaining biomass decreased slowly.

On Day 11 (decay stage), 2nd- and 3rd-instar maggots of *Ch. megacephala* and *M. domestica* were found at the anus region where the guts were exposed for both carcasses. Maggots were observed and collected daily from Day 11 (decay stage) up to Day 19 (remains stage) (Figure 4.50). Maggots of *Ch. megacephala* and *M. domestica* were found on Day 11, indicating that these maggots were able to survive in the viscous medium resulting from liquefication of fats degradation. However, fewer maggots colonised the carcasses in comparison to the carcasses untreated with insecticide. Lesser flies visited the carcasses during this period, probably the viscous medium in the carcasses were not attractive to the adult flies for oviposition.

Table 4.31. Dead fauna (adults) obtained on and around the monkey carcasses treated with malathion in a forested area.

Fauna	Order	Family	Species
Flies	Diptera	Calliphoridae	*Ch. megacephala*
			Ch. rufifacies
			Ch. velleneuve
			Ch. nigripes
			Ch. chani
		Muscidae	*O. spinigera*
			O. chalcogaster
			M. domestica
		Lauxaniidae	Unidentified sp.
		Platystomatidae	*Euthyplatystoma* sp.
		Dolichopodidae	Unidentified sp.
		Sarcophagidae	Unidentified sp.
Beetles	Coleoptera	Staphylinidae	*Aleochara* sp.
		Scarabaeidae	*Onthophagus penicillatus*
Ant	Hymenoptera	Formicidae	*Odontoponera* sp.
Cockroach	Blattodea	Blattellidae	*Blatella germanica*
Mite	Mesostigmata	Laelapidae	*Haemolaelaps* sp.
Butterfly	Lepidoptera	Nymphalidae	*Euploea mulcibar*

Advanced decay stage (Duration = 2 Days). This stage was noticed when majority of the bones and cartilages were observed. The inner organs and tissues under the skin were consumed by the maggots. However, the skins or external regions of the

carcasses remained intact. The odour of fermented material begun to reduce. Percentage of remaining biomass decreased rapidly. No liquefication was observed at the end of this stage. *Hermetia illucen* adults visited the carcasses on Day 16, but no oviposition was observed on the carcasses. *Ophyra spinigera* maggots were recovered from the carcasses from Day 15 (advanced decay stage) till Day 19 (remains stage) where bones were exposed.

Remains stage (Duration = 4 Days). The remains stage of the carcasses were bones, cartilages, hairs, skin layers and fermented tissue in a viscous medium. Very few maggots were observed on the carcasses. Less odour was noticed at this stage. Percentage of remaining biomass decreased very slowly until it stabilised. A total of 45.21% remaining biomass was observed because the majority of the skin layers and fermented tissue in viscous medium was not consumed by the maggots. Beetles and ants were found alive on the carcasses on Day 19, indicating that malathion had degraded on the monkey carcasses by then. No maggots were observed after Day 19 onwards.

Recent research (Goff & Lord, 1994; Introna *et al.*, 2001) has also demonstrated that the presence of drugs and/or toxins in decomposing tissues may alter the rate and patterns of development in arthropods using such tissues as food, thus potentially altering estimates of the PMI. The bloating stage was about 5 days, which is longer in comparison to carcasses without treatment with insecticide (Table 4.32), probably due to absence of maggot activities on the carcasses.

Based on our results, the 3rd-instar maggots of *Ch. megacephala* were obtained on Day 11. The development of *Ch. megacephala* maggots from 1st-instar to 3rd-instar took a minimum time of 72 hours (3 days) and the estimated period for eggs hatching after oviposition was about 24 hours (1 day). Thus, the estimated minimum PMI was 7 days. This study showed that PMI estimation on carcasses treated with insecticide was delayed by a minimum period of 7 days.

Table 4.32. Comparison on the duration of decomposition stages between monkey carcasses with and without treatment with insecticide placed outdoor in lowland forested area.

Decomposition Stages	Duration of Decomposition stages (Number of Day)			
	Carcasses treated with insecticide*		Carcarsses without treatment with insecticide*[#]	
	Duration	Mean Duration	Duration	Mean Duration
Fresh	2	2	2 – 3	2.5
Bloating	5	5	1 – 2	1.5
Decay	6	6	1 – 2	1.5
Advanced Decay	2	2	2	2
Remains	4	4	8 – 31	27.0
Total Duration of Decomposition	19	19	15 – 39	34.5
Duration of carcasses fully decomposed to remains stage	15	15	7 – 8	7.5

*2 replicates for each study
[#]Study 1 (Section 4.1.1) and Study 2 (Section 4.1.4)

Table 4.33. Comparison between the forensically important insects obtained from monkey carcasses with and without treatment with insecticide in lowland forested area in Gombak, Selangor, Malaysia. Only flies (Calliphoridae, Muscidae and Sarcophagidae), ants and beetles were shown in the list.

List of forensically important insect			Presence of forensically important insects	
Order	Family	Species	Carcasses treated with insecticide*	Carcasses without treatment with insecticide*[#]
Diptera	Calliphoridae	*Chrysomya chani* (Kurahashi)		+
		Chrysomya megachephala (Fabricius)	+	+
		Chrysomya pinguis (Walker)		+
		Chrysomya rufifacies (Macquart)		+
		Chrysomya villeneuve (Patton)		+
		Hypopygiopsis violacea (Macquart)		+
	Muscidae	*Musca domestica* (Linnaeus)	+	
		Ophyra spinigera (Stein)	+	+
	Sarcophagidae	Unidentified spp.		+
	Stratiomyiidae	*Hermetia illucens* (Linnaeus)		+
Hymenoptera	Formicidae	*Pheidologeton* spp. (Mayr)		+
		Odontoponera spp. (Mayr)	+	+
Coleoptera	Hybosoridae	*Phaeochroops rattus* (Arrow)	+	+
		Phaeochrous emarginatus (Laporte)	+	
	Scarabaeidae	*Onthophagus* nr. *Babirussa* (Eschscholtz)		+
		Onthophagus rudis (Sharp)		+
	Silphidae	*Chrysosilpha formosa* (Portevin)		+

*2 replicates for each study
[#]Study 1 (Section 4.1.1) and Study 2 (Section 4.1.4)

4.14 REFERENCES

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CHAPTER 5

DISCUSSION

5.1 THE CIRCADIAN ACTIVITY PATTERN OF BLOWFLIES ON MONKEY CARCASSES

Although a number of studies on nocturnal activity of blow flies on baits and carcasses were conducted around the world, most of these studies only reported the presence of fly activities by observing the occurrence of egg on the baits and carcasses. None of these studies reported on the timing of fly presence on the baits and carcasses quantitatively after sunrise and sunset, except a study conducted by Baldridge *et al*. (2006) in Texas. However, the sunrise and sunset time in Texas was different from this region.

Our study showed that when the monkeys were euthanised during nocturnal period, there was absence of oviposition by flies as observation was done hourly throughout the night. Flies were observed visiting the carcasses about half an hour after sunrise (mean = 29.25 ± 1.49 minutes) on the next morning until sunset. No fly was observed visiting the carcasses about 10 minutes after sunset (mean = 5.80 ± 0.68 minutes).

Our observation agreed with those of Baldridge *et al*. (2006), in which necrophilous flies were present and active at lighted and dark sites before and immediately after sunset, but fly activity on the bait (mice, beef and pork) ceased within 50 minutes post sunset and did not resume until next morning at 0600h.

Our observation was also consistent with studies conducted by Amendt *et al*. (2008). Their studies were conducted in different urban and rural locations in Frankfurt

and Munich, Germany by using dead hedgehogs and beef liver as baits in the field. Their results revealed that no nocturnal oviposition was observed in the field (Frankfurt field experiments: n = 28 and Munich field experiments: n = 23).

Zurawski *et al*. (2009) investigated the blow flies nocturnal oviposition in relation to sunrise and sunset in Michigan by placing pig carcasses in the field at 2 hours after sunset, and hourly observation was made into the following morning. Their study concluded that oviposition was never observed at night.

Furthermore, Haskell *et al*. (1997), Tessmer *et al*. (1995), Stamper & Debry (2007) and Stamper *et al*. (2009) also concluded that nocturnal oviposition of forensically important flies is an unlikely event.

A number of anecdotal observations suggest that nocturnal oviposition may occur, particularly with species of *Calliphora.* For example, *Calliphora vicina* females were observed laying eggs in a slaughterhouse at night (Green, 1951), and on a cadaver in a dark cave (Faucherre *et al*., 1999). However, experimental studies that have attempted to investigate these observations have given contradictory results. Nocturnal oviposition was observed in both *Calliphora vicina* and *Lucilia sericata* on carcass both placed directly on the ground (Greenberg, 1990) and on carcass raised off the ground to ensure that only flying insects were likely to reach it (Singh & Bharti, 2001). All these observations might be occurred under certain circumstances, such as high nightly temperatures, the presence of gravid flies with an appropriate arousal threshold and light intensity found on the sites as reported by Amendt *et al*. (2008) and Wooldridge *et al*. (2007).

Generally, calliphorid flies are considered to be inactive at night (Nuorteva, 1977; Erzinclioglu, 1996). As a result, a victim killed during the night would not be colonized before the following morning. This may lead to a considerable discrepancy

between the estimated post mortem interval (PMI) and the actual time of death of up to 12 hours.

Our observations conclude that diurnally active flies, such as calliphorids and muscids, do not oviposit during nighttime. These results should be considered when estimating the PMI in forensic entomological investigation.

5.2 FORENSIC ENTOMOLOGICAL STUDIES CONDUCTED IN OUTDOOR AND INDOOR CONDITIONS IN DIFFERENT ECOLOGICAL HABITATS IN MALAYSIA

There are several succession studies conducted in outdoor situations in Malaysia to establish baseline data to be used in estimation PMI (Lee & Marzuki, 1993; Omar *et al*., 1994a; Heo *et al*., 2007). However, such studies have never been conducted in indoor condition, and no studies have been conducted in coastal and montane areas as well. Hence, in order to fill up the gap of knowledge in this region, the present study attempted to determine and compare the arthropod succession associated with monkey remains in outdoor and indoor conditions. The insect succession and decomposition pattern of monkey remains are also discussed in this section.

It was interesting to note that 8, 3 and 5 species of calliphorid larvae were obtained from carcasses placed in lowland forested, coastal and montane forested areas (Figure 4.38, 4.39 and 4.40), respectively in this study. These flies are all of forensic importance and these flies have been recovered from human cadavers from the samples received from the hospital's Pathology Departments and Police Department (Lee *et al*., 2004; Syamsa *et al*., 2010). However, only *Lucilia porphyrina* larvae found on carcasses placed in outdoor and indoor conditions in montane forested area were never reported from any forensic entomological studies conducted in Malaysia. Among the

flies of the genus *Lucilia*, *Lucilia sericata*, *Lucilia illustris*, *Lucilia caesar*, *Lucilia eximia* and *Lucilia silvarum* were commonly reported in forensic entomological studies conducted in USA, Germany, Poland, New Zealand and Brazil, respectively (Sharanowski *et al*., 2008; Niederegger *et al*., 2010; Matuszewski *et al*., 2010; Eberhardt & Elliot, 2008; Carvalho *et al*., 2000), but not *Lucilia porphyrina*. None of these flies reported by the above researchers were recorded in this region as well. Thus, we believe that *Lucilia porphyrina* was exclusively found in montane forested area in tropical country, and can act as geographical indicator for corpses found in montane forested areas in this region.

In addition, most of the calliphoridae found in outdoor conditions also visited carcasses placed indoor, except *Chrysomya nigripes*, which could be a strictly outdoor species and will not invade cadavers in indoor conditions. We also noted that *Chrysomya nigripes* only invaded carcasses in lowland forested area. Another species of calliphoridae, *Chrysomya chani* was also found only invading carcasses placed in outdoor and indoor in lowland forested area, and a similar finding was reported by Omar *et al*. (1994a).

It is noteworthy to mention that adults and larvae of *Hypopygiopsis violacea* were often found on the carcasses during early decomposition stages in both lowland and montane forested areas. No pupae of this species were recovered from any monkey carcasses in this study. These larvae were observed to leave the beef and had the long post-feeding phase and pupation stage, as reported in our laboratory life cycle study (Chen *et al*., 2011). Thus, it was not surprising that this species was not found on human cadaver due to its behaviour. However, larvae of *H. violacea* was reported for the first time on human cadaver in Malaysia by Firdaus *et al.* (2010).

Lord *et al*. (1993) stated that the presence of *Hermetia illucens* in a cadaver can be of paramount importance in determining PMI in advanced decay decomposition

stage. A marked information from this study is that *Hermetia* larvae were only found on monkey carcasses placed outdoor and indoor in remains stage. This is noteworthy and is in line with the findings of Lord *et al*. (1993) that *H. illucens* will only be attracted to remains of cadaver after 20 to 30 days after death has occurred. This information is pivotal in the determination of PMI in Malaysian forensic cases because *H. illucens* larvae were often received for PMI determination (Nazni *et al*., 2011).

Mummification of carcass was observed during the advanced decay stage in outdoor and indoor conditions in all study sites, except carcasses placed outdoor in lowland forested area. although, Introna & Campobasso (2000) reported that Diptera do not oviposit on mummified tissues, this was in contrast with the present study where 12 dipteran species were recovered from the carcasses during mummification and remains stage, such as *Ch. chani*, *Ch. megacephala*, *Ch. nigripes*, *Ch. rufifacies*, *Ch. villeneuve*, *Hemipyrelia* spp., *Lucilia porphyrina*, *Fannia canicularis*, *Fannia scalaris*, *Musca domestica*, *Ophyra sinigera* and *Hermetia illucens*. This may be due to the fact that carcass decomposition duration and process was short in tropical countries, such as Malaysia, in comparison to temperate countries. For example, carcass decomposition duration in New Zealand (Eberhardt & Elliot, 2008), Argentina (Centeno *et al.*, 2002) and Colombia (Segura *et al.* 2009) took up to 126 days at mean temperature between 11ºC – 21ºC, more than 129 days at mean temperature at 19ºC, and 97 days at mean temperature of 14ºC, respectively.

The diversity of insect collected from outdoor and indoor in all study sites exhibited that Diptera had a peak during the initial stages of decomposition, and decreased at later stages. However, this was in contrast to coleopterans obtained from each stage, in which the number of beetle species collected from monkey carcasses placed in outdoor and indoor increased from fresh stage to remains stage. Our finding showed a similar pattern in insect succession as reported by Wolff *et al*. (2001), where

the flies are first necrophagous wave and they are the first to oviposit and the first immature stages collected. On the other hand, ants (Hymenoptera: Formicidae) were often seen visiting the carcasses in all decomposition stages in all ecological habitats.

Carcasses placed outdoor and indoor underwent 5 decomposition stages, namely fresh, bloating, decay, advanced decay and remains / dry as documented by Vitta *et al*. (2007), Bharti & Singh (2003), Arnaldos *et al*. (2004), Wolff *et al*. (2001) and Tatawi & El-Kady (1996). Our studies indicated that each decomposition stage in indoor condition was prolonged, compared to outdoor (Figure 4.37). The total decomposition duration on carcasses placed in indoor was prolonged, in comparison to outdoor in lowland forested and coastal areas by 1.67 to 2.73 folds, but this was not observed in montane forested area. According to our results, there was no significant difference in temperature and relative humidity between outdoor and indoor in the respective study site. Thus, the prolongation of decomposition duration of carcasses placed in indoor compared to outdoor may be due to the effects of rain encountered during the study period. Archer (2004) discovered that rainfall increased both mass loss rate and decomposition stage progression rates of exposed neonatal remains. In addition, Reibe & Madea (2010) reported that rain did not prevent the flies from ovipositing, either the flies waited for dry moment or they could reach the carcass by leaving the place of shelter and crawl towards the carcass. Reibe & Madea (2010) also reported that no correlation between rainfall and oviposition. Their results concluded that even if it is raining, the flies will oviposit on the same day of exposure of the outdoor carcass. Furthermore, Reibe & Madea (2010) also observed that whenever rain reached the carcass, the feeding maggots crawled beneath the piglet and fed on the moist tissue. Thus, the decomposed portions of carcasses placed outdoor in our study might have received more moisture and promote fly larvae feeding on it, compared to indoor.

On other hand, decomposition duration on carcasses placed outdoor and indoor in montane forested area only had 2 days difference between 2 conditions, and it could be considered as no obvious differences in decomposition duration on carcasses placed outdoor and indoor in montane forested area. This may be due to the low temperature in the mountain that affected the fly activity. This phenomenon was supported by Reibe & Madea (2010), in which low fly activity was observed at low temperature.

The carcasses located in study site with high ambient temperature [coastal area (mean temperature = 30ºC – 33ºC)] decomposed faster than those located in study sites with low ambient temperature [lowland forested area (mean temperature = 25ºC – 26ºC) and montane forested area (mean temperature = 20ºC – 21ºC)]. Our study was in line with Sharanowski *et al*. (2008) and Centeno *et al.* (2002). Sharanowski *et al*. (2008) reported that pig carrions were totally decomposed by 63 days in spring (mean temperature = 15.44ºC and 17.83ºC) and by 43 days in summer (mean temperature = 19.47ºC and 19.08ºC). Centeno *et al.* (2002) reported that pig carrions took up to 129 days to become decomposed in fall (mean temperature = 18.73ºC and 18.79ºC), but only took 22 days to become decomposed in spring (mean temperature = 29.50ºC and 31.87ºC) and summer (mean temperature = 40.75ºC and 41.75ºC). Their results indicated that carrions located in warmer climate were decomposed faster than in cold climate.

Our study revealed that flies visited shortly after the monkeys were euthanised and placed outdoor in all study sites. Oviposition was observed on the carcasses within a few hours in the daytime. This phenomenon was also observed by Heo *et al*. (2007) and Omar *et al*. (1994a).

However, when the monkeys were euthanised at nighttime and placed outdoor, fly oviposition was only observed after the sunrise of the next day. Thus, the PMI estimation was delayed by at least 12 hours.

It is interesting to note that there was at least 1 day of delay on the PMI estimation on carcasses placed indoor in coastal area, while a minimum of 3 days delay in PMI estimation was observed on carcasses placed indoor in both lowland and montane forested areas. The differences on delay on PMI estimation on carcasses may be due to the high temperature in coastal area that promoted bacterial activity in the stomach region during bloating stage, and producing smell to attract the flies to enter indoor and oviposit. This has been reported by Reibe & Madea (2010). However, they concluded that PMI estimation for corpses found indoor (mean temperature ranged between 17.8ºC and 23.9ºC) was only delayed up to 24 hours after exposure.

Corpses in houses or apartments are frequently found in late stages of decay infested by larvae of Calliphoridae, Phoridae, Muscidae or Sarcophagidae. Such persons are usually socially isolated, leading to delayed discovery of their body (Archer *et al*., 2005). For body found indoors, the presumed PMI often ranged between days and weeks as nobody discovered the corpses immediately after death (Reibe & Madea, 2010). Faucherre *et al*. (1999) reported that bodies can be colonized by insects in several different locations, including poorly accessible environments (Goff, 1991). In such cases, PMI estimation is complicated since it is unclear how promptly the blow flies found the body located indoors and started laying eggs (Reibe & Madea, 2010).

Delayed fly oviposition on corpses found in indoor located in different ecological habitats could broaden the range of PMI values if not fully understood. Our study revealed the time needed for blow flies to perceive the body and enter indoor to oviposit in three different ecological habitats. Hence, our results established how much time has to be added to estimate larvae age to arrive at a realistic PMI estimation in this region for the first time.

5.3 FIRST REPORT OF THE SIGNAL FLY, *SCHOLASTES* SP. (LOEW, 1873) (DIPTERA: PLATYSTOMATIDAE) ON ANIMAL CARCASSES IN MALAYSIA

Decomposition of carcasses was determined by five stages namely fresh, bloating, active decay, advanced-decay and dry/remains. Our study observed that *Scholastes* spp. (Diptera: Platystomatidae) visited the animal carcasses within an hour after death, which is the fresh stage of decomposition. This indicated that *Scholastes* sp. was attracted to freshly dead carcasses.

Platystomatidae, recently termed signal flies, belongs to order Diptera. Platystomatidae are worldwide in distribution and one of the largest families of acalyptrate with around 119 known genera and nearly 1,200 described species (McAlpine, 2001).

Some adult flies of Platystomatidae have been recorded by McAlpine (1973) attracted to flowers, decaying fruit, excrement, and decomposing snails. In our study, we observed that *Scholastes* sp. was attracted to dead animal and decaying fruit. This result was in line with McAlpine (1973) who claimed that this fly was attracted to decomposition in nature.

McAlpine (1973) stated that adult Platystomatidae are attracted to flowers, decaying fruit, excrement, and decomposing snails. In this study, we observed that *Scholastes* sp. was attracted to dead animal. According to Steyskal (1971), immature stages of Platystomatidae are found on fresh and decaying vegetation, carrion, human corpses, and root nodules of legumes. However, in our study, we did not find any larvae stages of *Scholastes* sp. feeding on monkey carcass and there is no evidence in literature to suggest that they are commonly found on decaying bodies of humans or other larger mammals.

The larva of an Australian species of the genus *Euprosopia* has been observed eating a coleopterous pupa, while the larvae of *Elassogaster linearis* (as *sepsoides*) have been recorded attacking egg pods of *Locusta migratoria* in the Philippines and Papua New Guinea, and *Trigonosoma decorum* (de Meijere) has been recorded feeding on human lesions (Steyskal 1971). So far, there is no report on Malaysian species of Platystomatidae playing a role as a predator for other pest insects or of any known medical importance. Thus, more ecological studies should be carried out to investigate the importance of Malaysian species of Platystomatidae as a new biological control agent and its medical importance.

It is possible to utilize the presence of signal fly, *Scholastes* sp. as a forensic indicator in determination of post-mortem interval (PMI), especially during the fresh stage of decomposition in Malaysia. However, it is also possible that this fly may be an incidental visitor to the animal carcasses. Their role in decomposition process still remains unknown.

5.4 FIRST REPORT OF THE HOUSE FLY LARVAE, *MUSCA DOMESTICA* (LINNAEUS) (DIPTERA: MUSCIDAE) ASSOCIATED WITH THE MONKEY CARCASS IN MALAYSIA

The housefly, *Musca domestica* (Linnaeus) (Diptera: Muscidae) is cosmopolitan all over the world and reported to exist wherever man has established himself (Omar *et al*., 2003). Adults and maggots of this fly can be found in fisheries, slaughter houses, vegetable farms, market places, garbage disposal sites and poultry farms (Bohart & Gressitt, 1951; Byrd & Castner, 2001; Nazni *et al*., 2003); while the oviposition of *M. domestica* on human corpses is rare (Greenberg, 1971; Smith, 1986).

In Malaysia, maggots of *M. domestica* were never reported from any entomological specimens from human corpses (Lee, per comm.). However, this was in contrast to the finding of Lectercq (1969) in Europe where the first wave of fly larvae in cadavers was inclusive of *M. domestica*. Reviews on forensic entomological cases of human corpse in Malaysia by Lee (1989, 1996), Hamid *et al.* (2003), Lee *et al*. (2004) and Salleh *et al*. (2007) showed that there was no evidence of infestation by *M. domestica* maggots on human cadavers so far. This may be due to the corpses were discovered with PMI less than 10 days, and during this duration, *Ch. rufifacies* maggots are predaceous in early decomposition stages, and frequently attack other maggots living in the same medium, resulting absence of *M. domestica*.

On the other hand, Vitta *et al.* (2007) and Heo *et al.* (2007, 2008a) reported adults of *M. domestica* visiting the pig carcasses (*Sus scrofa* Linnaeus) on the early stages (fresh stage and bloating stage), but no maggots of *M. domestica* were recovered from their studies. Heo *et al.* (2008b) reported the occurrence of *M. domestica* eggs on pig carcass (*Sus scrofa* Linnaeus) but no maggots were found from their study. Our study is the first record of *M. domestica* larvae recovered from a primate carcass in Malaysia.

The third-instar larvae of *M. domestica* were only found on Day-33 (remains stage) of a decomposed monkey carcass. No first-instar, second-instar and puparia of *M. domestica* were recovered from the carcass. Our study showed that *M. domestica* maggots were recovered together with another muscid fly maggots, *Ophyra (=Hydrotaea) spinigera* Stein on dry stage of a carcass. *Musca domestica* maggots were not obtained after this. *Ophyra spinigera* maggots were the dominant colonizer in the dry stage, and the occurrence of *M. domestica* may cause interspecific competition between both species of maggots. However, no study has been conducted on interspecific competition between these two species. According to Smith (1986),

Ophyra maggots are predaceous in the second and third instars, and frequently attack other maggots living in the same medium including *M. domestica* and other Muscidae. Only the occurrence of the third instar *M. domestica* observed in this study might probably be due to the predation by *O. spinigera*, thereby accounting for the complete absence of the maggots thereafter.

The occurrence of *O. spinigera* maggots in the decomposition stage of corpse and/or carcass is varied. As in this study, this fly was found associated with monkey carcass in the decay to dry stage. Smith (1986) reported that *Ophyra* usually appears in human corpses during the period of ammoniacal fermentation (decay), and 4 – 8 months (dry) after death. Besides, Byrd and Castner (2001) also reported that other species of *Ophyra* (*O. aenescens* Wiedemann and *O. leucostoma* Wiedemann) maggots was usually appeared during the late or active decay stages on human cadavers. In Thailand, the third instar larva of *O. spininera* was collected from the mummified human corpse, 3 – 6 months of decomposition (Sukontason *et al.*, 2001a). On the other hand, Omar *et al*. (1994a) reported that *O. spinigera* as a major colonizer of monkey carcasses when the carcasses were already in decay stage, 3 to 6 days after the carcasses were placed. Our finding was similar to the previous works (Sukontason *et al.*, 2001b; Heo *et al.* 2008a) that the third-instar larvae of *O. spinigera* were found from the carcass on decay and advanced decay stage (~ day 7 – 8th).

Heo *et al.* (2008b) reported the oviposition of *M. domestica* eggs on fresh pig carcass and concluded that this species maybe an early visitor. This contrasted with our study in which the *M. domestica* maggots were only observed on remains stage. Thus, the occurrence and role of *M. domestica* on forensic entomological study remain unclear. More studies should be conducted to investigate the role of *M. domestica* as an indicator in forensic entomological study.

5.5 ANTS (HYMENOPTERA: FORMICIDAE) RECOVERED FROM FORENSIC ENTOMOLOGICAL STUDIES CONDUCTED IN DIFFERENT ECOLOGICAL HABITATS IN MALAYSIA

The feeding habits of insects may range from being predatory, feeding on larvae and adults of other insects to necrophagous when insects consume exudates or decomposing tissues. Generally, ants feeding on carcasses are categorised as omnivorous species (Tabor *et al.*, 2005) in forensic entomological study. They can feed on both the corpse and associated fauna. Ants feeding on corpses can cause small punctate or scratch type lesions over the skin surface, which can be easily misinterpreted as ante-mortem abrasions or resulting from strong acids (Campobasso *et al*., 2009). However, ants are seldom reported as forensic indicators by crime scene investigators and forensic pathologists. Our study documented ants as common and potentially important indicators of ecological habitat in primate carcass experiments in Malaysia.

Ants were attracted to the blood of the freshly killed monkeys within 15 minutes of death. Heo *et al.* (2007) reported that flies in the family Muscidae were the first to be attracted to pig carcasses, followed by ants and spiders; this study occurred inside an oil palm plantation located in Tanjong Sepat, Selangor, Malaysia. Vitta *et al.* (2007) also reported ants as the first arthropod to be attracted to the wounds around the necks of the pig carcasses. However, Heo *et al*. (2007) and Vitta *et al*. (2007) did not identify the ant specimens visiting the pig carcasses in their studies.

Ants were observed in all the decomposition stages of the carcasses. These ants were observed to actively prey upon the eggs, maggots, pupae and newly emerged flies throughout the study period. Our findings were similar to that reported by Campobasso *et al*. (2009) in which ants were present at all stages of decomposition as they are typically observed shortly after death or during the early postmortem period, but even

later once the fly maggots had left the carrions. Both studies observed ant activity shortly after death and during the early stages of decay; this ant activity continued even after all fly and maggot activity ceased. Additionally, ants also have been reported as predators in the context of forensic entomology by Smith (1986). Our results revealed that *Tetramorium* sp. was only found visiting carcasses placed indoors; while *Oecophylla smaragdina*, *Cardiocondyla* sp., *Hypoponera* sp. and *Pachycondyla* sp. were only found on carcasses placed outdoors. However, no specific references were reported on the foraging behaviour and localities preferences of ants obtained from forensic entomological studies conducted in indoor and outdoor.

Campobasso *et al*. (2009) reported that the main role of ants in faunal succession varied from predators on the eggs and larvae of other insects (mainly Diptera), thus reducing significantly the rate of decomposition; to scavengers on the flesh or exudates from the corpse itself. Ants have been shown to negatively impact the occurrence and development of forensically significant flies on the corpse, the presence or absence of ants should be taken into account in every case involving postmortem interval estimates based on entomological evidence. Furthermore, ants can feed on both the corpse and associated fauna. Thus, ants feeding on the carcasses also could hasten the loss of carcass mass (Campobasso *et al*., 2009). However, we did not study the relationship between decomposition rates of the carcasses and the presence of ants in this study.

Ants were also reported to play an important role in inflicting postmortem injuries to the carcasses (Moura *et al*., 1997). In this study, ants were found to be attracted to the bloodstains and fly eggs on the fresh carcasses, but no lesions were observed to be caused by ants. According to Campobasso *et al*. (2009), the feeding action of ants could cause many irregular, serpiginous, scalloped areas of superficial skin loss, and small punctate and scratch-type lesions may be observed on the body, which were results of postmortem ant bites. These injuries consisted of small and rather

shallow gnawed holes that could be easily misinterpreted as antemortem abrasions or resulting from strong acids.

In this study, ants were observed occurring in all the decomposition stages, indicating that ants were not a significant indicator for carcasses succession or post mortem interval estimation. However, we found that different species of ants visited the monkey carcasses placed in different ecological habitats (Table 4.22). *Cardiocondyla* sp. was only found on carcasses placed in the coastal area; while *Pheidole longipes*, *Hypoponera* sp. and *Pachycondyla* sp. were found on carcasses placed in the highland area. Compared to study conducted by Heo *et al*. (2009a), only *Anoplolepis gracilipes* (Smith, 1857) and *Diacamma* sp. (Mayr, 1862) were found on pig carcasses placed in oil palm plantations. On the other hand, *Pheidologeton diversus*, *Tetramorium* sp. and *Odontoponera transversa* found on carcasses placed in the forested area were also found in the oil palm plantation as reported by Heo *et al*. (2009a). We also noticed that some of the ants were found in more than one ecological habitat, e.g. *Oecophylla smaragdina* in both coastal area and oil palm plantation, *Paratrechina longicornis* in both coastal and highland areas, *Odontoponera transversa* in both forested areas and oil palm plantations, and *Pheidologeton diversus* in all ecological habitats surveyed except highland areas. It is possible that some ant species can be used as geographical indicators while other species are too widely distributed to indicate a specific locality.

Goff and Win (1997) also reported the combined use of forensically important flies and ants to estimate the PMI. In their study, human remains were discovered in a metal box. Both ants and flies were used to estimate the PMI. The developmental time of the stratiomyid fly, *Hermetia illucens* (Linnaeus, 1758) was used in conjunction with the time required for ants, *Anoplolepsis gracilips* to establish a colony capable of producing alate (winged) reproductives.

Ants collected during this study of monkey carcasses in Malaysia may play an important role in faunal succession and may also be capable of skewing decomposition rates. Preliminary findings indicate that ants may serve as an indicator of geographic locality; this information could aid crime scene investigations in the future. More studies should be conducted in different locations to obtain more accurate information to determine the precise role of ants in forensic investigation.

Additional studies of ant behavior and distribution will further add to the body of knowledge used by forensic investigators. The life cycles of different ant species should be studied in a manner similar to that of the forensically significant flies using developmental data based on degree day accumulation. It is possible that a more accurate estimation of PMI using ants could be developed with increased knowledge. A comparative study on faunal succession in the presence and absence of ants also should be studied in order to investigate the role of ants in PMI estimation in this region.

5.6 BEETLES (COLEOPTERA) RECOVERED FROM FORENSIC ENTOMOLOGICAL STUDIES CONDUCTED IN DIFFERENT ECOLOGICAL HABITATS IN MALAYSIA

The beetles comprise 25% of all animal species described to date and they are found in a wide diversity of habitats and exhibit a range of lifestyles, including colonization on corpses (Gunn, 2006). The beetles are significant groups of insects in forensic studies. The main families are Silphidae, Staphylinidae, Scarabaeidae, Carabidae, Histeridae and Dermestidae (Goff & Catts, 1990).

Most of the forensic entomological studies were focused on Diptera, especially flies belonging to the families of Calliphoridae, Muscidae and Sacrophagidae. This is probably because these flies are able to locate the corpses faster and they can provide a

more accurate estimate of minimum post mortem interval (Midgley *et al*., 2010). However, our study revealed that *Phaeochroops rattus* (Coleoptera: Hybosoridae) can locate the monkey carcasses as early as in the fresh stage (Day 3 of death). Midgley & Villet (2009) reported that *Thanatophilus micans* (Coleoptera: Silphidae) was able to locate corpses and start breeding within 24 hours of death. Early & Goff (1987) also reported that adult beetles can be collected from as early as 3 – 5 days after death and 10 days after death but larvae were not reported. They concluded that the potential utility of beetles for post mortem interval estimation is equal to that based on flies (Midgley & Villet, 2009; Early & Goff, 1987).

Our study revealed that more Coleopteran species were found in indoor compared to outdoor, in contrast to the study reported by Goff (1991). However, similar observation was reported in forensic entomological studies conducted in forested area by Nazni *et al*. (2011).

Our findings recorded 5 species of beetles belonging to the family of Hybosoridae (*Phaeochroops rattus*, *Phaeochroops peninsularis* and *Phaeochrous emarginatus*) and Scarabaeidae (*Onthophagus* sp. nr. *babirussa* and *Onthophagus rudis*) only visited carcasses placed in lowland forested area; while 7 species of beetles belonging to the family of Scarabaeidae (*Sericania* sp.), Staphylinidae (*Atheta* sp., *Creochara brevipennis*, *Hesperus* sp., *Ontholestes* sp. and *Philonthus* sp.) and Leiodidae (*Nemadus* sp.) were only found in carcasses placed in montane forested area, indicating that beetles can serve as an indicator of geographic locality. According to Midgley *et al*. (2010), use of the forensically important beetles for post mortem interval estimation will differ from region to region.

According to Byrd & Castner (2001), post mortem interval estimation can be based on the duration of the immature stages of the insects found on a corpse or on the community composition of insects on the corpse. The duration of the immature stages is

generally longer in beetles compared to flies, which means that beetles are useful to estimate post mortem interval not only during early decomposition, but also in later stages of decomposition (Midgley *et al*., 2010). However, our study only recorded immature larvae of *Chrysosilpha formosa* (Silphidae) and *Philonthus* spp. (Staphylinidae) at late decomposition stages (advanced decay stage and remains stage) of monkey carcasses. This may be because carcass decomposition duration and process was short in tropical countries, such as Malaysia, in comparison to temperate countries. The decomposition process was shortened due to high temperature which resulted in speedy development of immature of flies and rapid decay of carcasses. Under these conditions, many fly larvae would have left the carcasses for pupation. This will result in lack of suitable medium (decayed organic matter) and food sources (immature of flies) for colonization of beetles. Kulshrestha & Satpathy (2001) reported that the mere presence of a few beetles on corpse did not represent the actual species infestation on remains. In addition, it is likely that the beetles might have come from a nearby habitat and only the presence of larvae colony represented an actual species infestation that could be used for post mortem interval (Kulshrestha & Satpathy, 2001).

The most accurate method of estimating post mortem interval using insects is to use models based on development of immature stages (Higley & Haskell, 2001). The data on the development of immature forensically important flies has been well documented and refined as models for post mortem interval estimation in Malaysia. However, this was not done on any Malaysian forensically important beetles. Thus, the utility of beetles in post mortem interval estimation is still under consideration, and further study is required to develop the statistically robust models.

Identification of necrophagous beetles is not easy because many of them are neither cosmopolitan nor pestilent (Midgley *et al*., 2010). Their identification depends on the taxonomic advancement of broad geographic areas in which the corpses are

located. Taxonomic keys for forensically important beetles have been developed in many countries, such as Silphidae which can be identified using keys of Schawaller (1981; 1987) and Peck (2001), Histeridae using Caterino & Vogler (2002), and Staphylinidae using Solodovnikov & Newton (2005). All these taxonomic keys can provide easy identification of beetles in a given area / countries, but should be used with caution outside the geographic range treated.

Although Malaysia is a mega biodiversity country, the discovery of unidentified beetle species is still in progress. Furthermore, taxonomic key for forensically important beetles has not been developed in this country. In this study, we have identified the families and species of Coleoptera visiting monkey carcasses in lowland and montane forested areas in Peninsular Malaysia, and their distribution over the decomposition stages. This is the first study done in Malaysia on the Coleoptera succession on carcasses. Data obtained from this study may help to interpret the Coleoptera evidence in forensic cases occurring in similar ecological habitats. However, beetles colonization on corpses may vary according to geographical regions. Hence, more forensic entomological studies related to beetles succession should be conducted to enhance the knowledge on this matter.

5.7 FAUNA SUCCESSION AND DECOMPOSITION ON INCINERATED MONKEY CARCASSES

Forensic entomological studies on insect species found on exposed human corpses (Lee, 1996; Lee *et al*., 2004) and animal remains (Lee & Marzuki, 1993; Omar *et al*., 1994a; Heo *et al*., 2007; Azwandi & Abu Hassan, 2009) have been well reported in Malaysia. The knowledge on insect succession pattern has been used by researchers / forensic entomologists in post mortem interval (PMI) estimation in cases of homicide, suicide,

accident and unattended death due to natural causes in this country since 1951 (Lee *et al*., 1984; Hamid *et al*., 2003; Lee *et al*., 2004; Salleh *et al*., 2007).

In some cases, forensic entomological analysis sometimes requires the individualization of incinerated bodies of which only some parts of body remains are available. Furthermore, in the assessment of PMI on incinerated bodies the question of whether the incineration effects deter or promote the arrival and oviposition activity of forensically important insects, especially blowflies is of crucial importance. Little information has been published on insect succession on incinerated human corpses and animal remains in Malaysia.

Reports on occurrence of maggots in incinerated human cadavers were relatively rare in Malaysia (Lee, per comm.). Recent study by Heo *et al*. (2008a) showed that there was no significant difference between the rate of decomposition and sequence of faunal succession on partially burned and unburned pig (*Sus scrofa* Linnaeus) carcasses. However, Heo (per comm.) reported that only about 50% of the pig skin was charred in his study. No study was reported on highly burned remains in this country.

In order to obtain more useful information regarding insect succession on incinerated human remains, this study simulated a situation in which victim had been deliberately burned to conceal the crime, or those in which cremation has resulted from an accident or suicide. The present study was carried out to investigate the effect of incineration on the PMI estimation and insect succession on badly incinerated monkey (*Macaca fascicularis*, Raffles) carcasses in a forested area in Gombak, Selangor, Malaysia.

Murderers often attempt to dispose off their victim's body by burning the corpse. However, they are seldom completely successful owing to the extremely high temperatures required. Bohnert *et al*. (1998) reported that complete incineration of human body to become ashes produced by a crematorium took about 2 – 3 hours at

temperatures between 670 and 810°C. Furthermore, even in the extreme heat of a crematorium or badly burned bodies found in house fire and traffic accidents, recognizable pieces of human remains were still present (Kennedy, 1996; Dedouit *et al*., 2007; Schwark *et al*., 2010). For example, there was a murder case in Taiwan, a girl was burned and found on a side path close to a sugar cane field, but the PMI was estimated successfully according to calliphorines obtained from the corpse (Pai *et al*, 2007). In another two cases in Italy, time of death of both burned victims were also determined by using insect evidence (Introna *et al*., 1998).

Hypopygiopsis violacea and *Ch. megacephala* were earlier visitors to the carcass 30 minutes after incineration. This was similar to our finding on non-incinerated carcasses in Section 4.1.1, in that *H. violacea* was noticed as first visitor to the carcasses within 1 hour after the monkey was euthanized. This study showed that there was no difference on the arrival and oviposition activity of flies on the freshly incinerated and non-incinerated carcasses, indicating incineration effect did not deter the arrival of flies. Lee (per comm.) examined the maggots recovered from case of partially burnt and decomposed corpse found in the jungle and noted the presence of both *Ch. megacephala* and *Ch. rufifacies*. He further observed that these maggots seemed to develop normally and not affected by the incineration. Omar *et al*. (1994b) reported that *H. violacea* arrived within 1 hour after the fresh monkey carcasses were placed in a forested area. Pai *et al*. (2007) found that the blow flies (Calliphoridae) had arrived on burned pig carcass within 5 minutes after placing the carcass in the study site. They also noticed that most of the flies visiting that carcass were *Ch. megacephala*.

Chrysomya villeneuve and *Ch. chani* were dominant adults and larvae obtained from monkey carcasses in decay and advanced decay stages; while *O. spinigera* was dominant in the remains stage. The larvae of *Ch. villeneuve* occurred later than *Ch. megacephala* and *Ch. pinguis* on the carcasses, this might be due to their aggressive

feeding habit of 2nd- and 3rd-instar larvae on other fly larvae (Sukontason *et al*., 2006). However, *Ch. villeneuve* and *Ch. chani* were finally replaced by another predacious larvae, *O. spinigera*. *Ophyra spinigera* has been reported predaceous in the second and third instars, and frequently attack other maggots living in the same medium (Smith, 1986; Chen *et al*., 2010) and predating pupae of *Ch. rufifacies* (Heo *et al*., 2009b).

According to Catts & Goff (1992), the oviposition of flies was deterred by burning, but this no doubt depends on the level of burning and incineration. In this study, the carcasses were incinerated to give a Crow-Glassman Scale (CGS) (Table 4.24) at level 2 with 100% body surface / skin burned. The level of burning and incineration in this study (according to CGS described in Glassman & Crow, 1996) was not similar to study by Heo *et al*. (2008a), in which only about 50% of body surface / skin was incinerated (Heo, per comm.). Their findings suggested that blowflies were not attracted to freshly burned pig carcass on the first day contrasted our study. This might be due to the presence of fuel (petrol) on the carcass and incomplete combustion of the fuel. Thus, the remaining fuel deterred the arrival of flies. Marchenko (2001) reported that the time for insects to invade the cadaver would be delayed if the cloths on the cadaver were stained with combustible materials. On the other hand, Avila & Goff (1998) reported that oviposition of calliphorines occurred one day earlier on burned carcass than the unburned carcass.

Generally, fly and ant species obtained from non-incinerated carcasses were more diverse than incinerated carcasses and vice versa for beetles (Table 4.26). However, Avila & Goff (1998) and Heo *et al*. (2008a) found that arthropod fauna which colonized the burned and unburned carcasses were basically the same. The beetle (Coleoptera) contains many families of significance in forensic studies. The main families among them being Staphylinidae, Scarabaeidae, Carabidae, Histeridae, Sliphidae and Dermestidae (Goff & Catts, 1990). In this study, only adult beetles

belonging to the family of Staphylinidae, Scarabaeidae, Histeridae and Sliphidae were obtained from the monkey carcasses.

The fauna succession pattern was similar in both incinerated and non-incinerated carcasses with the sequence from Calliphoridae, Sarcophagidae to Muscidae, indicating incineration effect did not affect the pattern of fauna succession. This was slightly different from study by Heo *et al.* (2008a), in which Phoridae and Stratiomyidae were found in the later stage of decomposition on a burned pig carcass. However, Heo *et al.* (2008a) concluded that the burning did not affect the sequence of fauna succession on burned and unburned pig carcasses.

Our study revealed that the decomposition rate of incinerated carcasses was faster compared to non-incinerated carcasses. Our findings generally agreed with studies conducted by Avila & Goff (1998) and Heo *et al*. (2008a). According to Avila & Goff (1998), the burned carcasses attracted much more fly oviposition than the unburned carcasses, showing that burned carcasses are still extremely attractive to calliphorine flies. Heo *et al*. (2008a) also reported that more larvae activities occurred on burned pig and hence generated more heat and thereby quickened the rate of decomposition. Nevertheless, it should be noted that if the estimated PMI of incinerated carcass was based on the decomposition stages occurring on a non-incinerated carcass, the estimation would be longer than the actual time.

According to Gunn (2006), burning sterilizes the skin surface and dries the underlying tissues, making them unsuitable for growth of microbes and blowfly maggots, but it also causes cracks through which they may invade the deeper tissues that are less affected. Moreover, the decomposition rate and fauna succession may differ depending on the level of burning and amount of incineration (Byrd & Castner, 2001). Thus, burning may reduce or promote the decomposition rate, and is difficult to generalize. Additional research is needed to ascertain the effects of various levels of

burning on rate of decomposition and succession of arthropods on the incinerated carcass in Malaysia.

In conclusion, this study emphasizes that (1) incineration effect does not deter the arrival and oviposition of forensically important flies on the carcasses; (2) incineration effect does not make any difference on the fauna succession pattern between incinerated and non-incinerated carcasses; and (3) decomposition rates on incinerated carcasses are faster than non-incinerated carcasses.

5.8 FAUNA SUCCESSION AND DECOMPOSITION ON MONKEY CARCASSES SUBMERGED IN FRESHWATER RIVER

In Malaysia, many researches done on the estimation of postmortem interval (PMI) by using animal carcasses had focused its interests on the process of decomposition and fauna succession in terrestrial environments, such as rubber plantation (Omar *et al*., 1994a), oil palm plantation (Heo *et al*., 2007; Heo *et al*., 2008a; Azwandi & Abu Hassan, 2009), secondary forest (Lee & Marzuki, 1993; Chen *et al.*, 2008; Chen *et al*., 2010) and urban area (Ikhwan, 2004). Although all these studies were very important and provided valuable information for forensic entomology aspect especially on PMI estimation, it leaves a forensic scenario open to questions. This scenario entails the decomposition of body submerged in aquatic environments.

Corpses recovered from different water bodies and aquatic environments are quite common in Malaysia (Lee *et al.*, 2004; Kavitha *et al*., 2008), but studies documenting this phenomenon are still uncommon. The forensic entomological studies and estimation of PMI in aquatic environments is complex, and has been studied and well documented by many researchers around the world (Payne & King, 1972; Haskell

et al., 1989; Davis & Goff, 2000; Hobischak & Anderson, 2002). However, forensic entomological studies in aquatic environments have only just begun in Malaysia.

In 2008, the study on the decomposition and insect succession on animal carcass in aquatic environment was published by Heo *et al*. (2008a) for the first time in Malaysia. Heo *et al*. (2008a) highlighted on the decomposition stages and insect activities on piglet carcass submerged in freshwater pond located in an oil palm plantation in Malaysia. Their study had generated useful information on fauna succession in freshwater pond in this country. However, the knowledge gaps on the decomposition rate and fauna succession in different type of aquatic habitats and water body in this region still remain unknown.

The present study attempts to investigate the PMI estimation and insect succession on monkey (*Macaca fascicularis*, Raffles) carcasses submerged in freshwater river located in a forested area in Gombak, Selangor, Malaysia.

Generally, carcasses submerged in freshwater river took about 9 – 11 days to reach floating remains stage, in comparison carcasses placed outdoor on ground only took about 7 – 8 days to reach remains stage (Table 2). The results indicated that the decomposition rate of carcasses submerged in freshwater was slower than carcasses placed outdoor by 2 – 3 days. Rodriguez (1997) reported that decomposition of submerged individual occurs at approximately half the rate of bodies that are outdoors because of the cooler temperatures and inhibition of insect activity. In addition, Simpson & Knight (1985) reported that human decomposition is slowed in water because the body temperature cools twice as quickly as it does in air. Moreover, the abundance of the decomposer especially fly maggots on carcasses submerged in water was also obviously lesser than carcasses placed outdoor. Based on our observation, the lack of maggots mass also acted as an important factor that affected the decomposition

rate in this study, and this observation was supported by MacDonell & Anderson (1997).

Heo *et al*. (2008a) reported that piglet carcass submerged in freshwater pond took 9 days to reach sunken remains stage, while our study showed that monkey carcasses submerged in freshwater river took 11 – 13 days to reach the similar stage. This variation may be due to two factors that affect the arrival of flies and decomposition rate. First, the piglet carcass placed in pond was exposed to sunlight directly, while monkey carcasses in this study were placed in a river located in secondary forest shaded by trees. The exposure to direct sunlight made the water temperature in standing water pond warmer, compared to a running water river. Second, the water temperature in Heo *et al*. (2008a) study (mean = 28.3ºC) was significantly higher than this study (mean = 21.4ºC) ($p < 0.05$). Rodriguez (1997) reported that decomposition process is highly variable because it is temperature dependent. Decomposition cause gases and bacteria to accumulate in the gut, which cause body to begin to rise to the water surface; and the warmer the water temperature, the quicker this process will occur (Rodriguez, 1997). Thus, insects will start colonizing the carcasses once it rises up to the water surface.

According to Payne & King (1972) and Haskell *et al.* (1989), submersion in freshwater environments can alter the terrestrial fauna succession on carcasses or corpses. Beside flies, no other insects were observed to visit the carcasses (Table 3). Our study revealed that the fly species on carcasses placed outdoor are more diverse, in comparison to carcasses submerged in water (Table 3). Furthermore, there were no ants and beetles found on carcasses placed in water. This is due to the carcasses were surrounded by running water and there was no shrubs or sticks connecting with the metal cage, thus avoiding the ants and beetles contact to the carcasses. This was supported by Campobasso *et al.* (2001), in which bodies in aquatic environments

prevent oviposition and subsequent development of terrestrial necrophagous insect larvae. However, Payne & King (1972) reported that beetles belonging to the family of Silphidae, Staphylinidae and Histeridae were observed on floating decay stage, copulating and preying on maggots colonized on pig carcasses.

The presence of aquatic insects and their subsequent contribution to the decomposition process and submersion intervals were absent in this study. According to Haskell *et al*. (1989), the midges (Diptera: Chironomidae) among the aquatic species most often associated to immersed bodies and they are extremely common in most freshwater situations. Keiper *et al*. (1997) detected midge colony on rat carcasses successional patterns. The diversity of genera increased after 29 days and larvae (Orthocladiinae) were dominant insects colonizing the carcasses followed by the subfamilies of Chironominae and Tanypodinae, the timeline observed can be applied to the determination of postmortem submersion interval.

Besides, aquatic predators also were absent in this study. Mottonen & Nuutila (1977) reported that in the natural milieu, aquatic predators (fish and crustaceans) would take part in the decomposition process by feeding and disarticulating on corpses. The damages that aquatic creatures inflict on a decomposed body in water increases the difficulty of assessing cause of death or determining identification of the individual (Mottonen & Nuutila, 1977).

Chrysomya megacephala was the dominant adults observed visiting the monkey carcasses in floating decay stage; however, none of maggots of this species was obtained from this study. Interestingly, *Ch. pinguis* and *Hemipyrelia* sp. were found as dominant species of maggots colonizing on carcasses submerged in freshwater river. The correlation between the oviposition preference / behaviour of *Ch. pinguis* and *Hemipyrelia* sp. and monkey carcasses submerged in water remains unknown. Our finding was in contrast with Heo *et al*. (2008a), in which the majority of fly maggots

observed on pig carcass submerged in freshwater pond were *Ch. megacephala*, followed by *Ch. rufifacies*. It may be due to different oviposition behaviour of these flies on different ecological habitats. According to Lee (per comm.), the larvae of *Chrysomya*, especially *Ch. megacephala* were found in five examples of forensic cases involving human cadavers found in water in Malaysia. Hence, it appears that *Chrysomya* maggots are commonly found in human cadavers under aquatic conditions.

Lee (per comm.) also mentioned in another case, the aquatic larvae of *Eristalis* species were found on the corpse of an infant floating on an irrigation canal. However, the larvae of *Eristalis* species were not obtained in this study. This may be due to this species of larvae were only colonized in water body containing high organic matter, while our study was conducted in fresh water river.

The ability for forensic pathologists, crime scene investigators and forensic entomologists to determine the PMI of a body is crucial to any death investigation. Further studies on the different aquatic ecological habitats (freshwater and marine) could result in more accurate estimation of decomposed body found in such environments in this country.

In conclusion, this study emphasizes that (1) PMI estimation on carcasses submerged in freshwater river was delayed by a minimum period of 2 days; (2) there was no difference on the fly succession pattern between carcasses submerged in freshwater river and placed outdoor, with the sequence from Calliphoridae, Sarcophagidae and Muscidae; (3) *Ch. megacephala* was the dominant adult flies observed to visit the carcasses, but only *Ch. pinguis* and *Hemipyrelia* sp. were found as dominant maggots colonizing on the carcasses; and (4) decomposition rates on carcasses submerged in freshwater river were slower than carcasses placed outdoor.

5.9 FAUNA SUCCESSION AND DECOMPOSITION ON MONKEY CARCASSES TREATED WITH INSECTICIDE

In some forensic cases, the murderer may attempt to confuse the evidence by treating the cadaver with a chemical that deters the arrival of forensically important insects such as blowflies for laying eggs. The chemical on the carrion may be absorbed into the body and influenced maggot development.

Malathion, an organophosphate has become one of the most commonly used insecticides, following the development of insect or pest resistance against DDT (an organochlorine) in many countries (Rozendaal, 1997). Generally, malathion is one of the safest insecticides because of its low toxicity to mammals. In rats, it has a low acute oral lethal dose with 50% lethal rate at 900 to 5,800 mg/kg, and it can be broken down by mammalian liver (Goff, 2001).

With its low toxicity to mammals, its ease of application, and ability to kill a wide variety of insects and mites, malathion has become a popular insecticide for home and gardens (Goff, 2001). Because of its availability, malathion is widely used in homes and gardens and becomes a common poison in suicide-related poisoning (Moriya *et al.*, 1991; Pahwa, 1991; Zivot *et al*., 1993; Casey & Vale, 1994; Thompson *et al*., 1998).

The present study was conducted to observe the effect of malathion on the PMI and succession of insect on monkey (*Macaca fascicularis*, Raffles) carrions in a forested area in Gombak, Selangor, Malaysia.

The carcasses used in this study were placed outdoor, in contact with the soil, and the surrounding area was forested and open to insect invasions. However, after the carcasses have been exposed for 11 days, then only we were able to find fewer species of larvae from the carcasses than expected on carcasses without treatment with insecticide at the same study site. It was surprising to note that the number of maggots

found on the carcasses was respectively low and scarce in comparison to carcasses without treatment with insecticide, in which maggots mass were found on and around the carcasses. This may be due to the decomposition processes of insecticide-treated carcasses producing a viscous medium which is not attractive for oviposition.

Normally, after the carcasses were exposed for about 1 week, it was possible to obtain a number species of maggots, particularly *Hypopygiopsis* sp., *Ch. villeneuve*, *Ch. rufifacies*, *Ch. chani*, *Ch. nigripes*, *Ch. pinguis*, *Ch. megacephala* and *Ophyra* sp. However, in the present study only 3 species of maggots, *Ch. megacephala*, *M. domestica* and *Ophyra* sp were found. Thus, we concluded that malathion repelled and delayed the insect invasions of the carcasses, especially flies for at least 7 days. After that, although malathion was still present, the concentration might not be high enough to repel and kill the flies to oviposit their eggs on the carcasses.

There were few reports on the effect of insecticide on maggots growth rates. Inoue (1964) reported the effects of malathion on maggots of flesh fly, *Boettcherisca peregrine* in which very high malathion concentrations were required to kill this maggots when the insecticide was applied directly onto the maggots' outer cuticle. His findings may explain the survival of *Ch. megacephala*, *M. domestica* and *Ophyra* sp. on the carcasses treated with malathion in this study. In addition, *Ch. megacephala* and *M. domestica* are normally associated with poultry farm and human dwelling, and they may have commonly exposed to the insecticides during control program. These two species of flies were more tolerance against insecticides and able to survive on monkey carcasses containing malathion in this study.

Beside, Lambiase *et al*. (2008) reported collected dead larvae from a human female corpse treated with insecticide, and identified them as 1^{st}, 2^{nd} and 3^{rd} instar larvae of *Lucilia caesar*, *Sarcophaga* sp. and *Chrysomya albiceps*. The results indicated the PMI was 8 – 12 days. However, the PMI was overestimated when compared to the

extent of decomposition of the corpse. It is thus obvious that the PMI was delayed by the effect of insecticide on the insect succession on the body, which is supported by the findings from this study.

Studies have also been done on detection of drug, toxins and insecticides in maggots in suicide cases by analysing the maggots which had fed on the corpse. According to Goff & Lord (1994) and Rodriguez & Lord (1993), insects obtained from decomposing corpse not only can be used in estimation of PMI, but also can serve as reliable alternate specimens for toxicological analysis in the absence of tissues and fluids normally taken for such purpose.

Recent research has also demonstrated that the presence of drugs and/or toxins in decomposing tissues may alter the rate and patterns of development in arthropods using such tissues as food, thus potentially altering estimates of the PMI (Goff & Lord, 1994; Introna *et al.*, 2001).

Gunatilake & Goff (1989) reported that a 58-year-old male with a history of attempted suicides found dead in a crawl space in Honolulu, Hawaii was last seen 8 days earlier. Two species of flies, *Ch. megacephala* and *Ch. rufifacies* were found on the corpse, and tissue samples from the body revealed presence of malathion. Investigators found it abnormal that, given the conditions, there were only 2 fly species found on the body and these species revealed a PMI of 5 days. Thus, it was determined that the presence of malathion in the corpse delayed the PMI for a few days.

Rashid *et al*. (2008) conducted a study on the effect of malathion on the growth rate of *Ch. megacephala* in malathion-exposed rat carcass. Their results indicated that malathion extended the development of *Ch. megacephala* to 10 days compared to 7 days in control colony. They concluded that the presence of malathion altered the development rate of *Ch. megacephala* and thus disrupted normal PMI estimation.

In order to estimate the PMI based on the duration of larval development on corpse, application of accurate larval growth data is crucial. Thus, more studies should be conducted to investigate the effect of various drugs, toxins and insecticides on the growth rates and growth parameters of various forensically important flies in this region.

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CHAPTER 6

CONCLUSION

1. No nocturnal activity and oviposition of forensically important flies during nighttime. Flies activity was observed about 30 minutes after sunrise and actively throughout the day until 10 minutes after sunset.

2. Oviposition of forensically important flies on carcasses not only can be used for PMI estimation, but also can serve as geographical indicator:
   (i) *Lucilia porphyrina* was exclusively found in montane forested area, and can act as a geographical indicator for corpses found in montane forested areas in this region.
   (ii) *Chrysomya nigripes* could be a strictly outdoor species and only invaded on carcasses in lowland forested area.
   (iii) *Chrysomya chani* was only found invading on carcasses placed in outdoor and indoor in lowland forested area.

3. Carcasses placed outdoor and indoor underwent 5 decomposition stages namely, fresh, bloating, decay, advanced decay and remains / dry. Each decomposition stage in indoor condition was prolonged compared to outdoor. Thus, total decomposition duration on carcasses placed in indoor was prolonged, in comparison to outdoor in lowland forested and coastal areas by 1.67 to 2.73 folds, but this did not occur in montane forested area. No obvious decomposition duration on carcasses placed outdoor and indoor in montane forested area.

4. At least a delay by 1 day on the PMI estimation on carcasses placed indoor in coastal area, while a minimum of 3 days delayed in PMI estimation on carcasses placed indoor in both lowland and montane forested areas were observed. This study revealed that the time required for blow flies to perceive the body and enter indoor to oviposit in three different ecological habitats. Our results established how much time has to be added to an estimated larvae age to approach a realistic PMI estimation in this region for the first time.

5. The signal fly, *Scholaster* sp. (Diptera: Platystomatidae) was collected during the fresh stage of decomposition in Malaysia. However, it is possible that this fly may be an incidental visitor to the animal carcass. Their role in decomposition process still remains unknown. This study reported the presence of signal fly, *Scholaster* sp. in forensic entomological study in Malaysia for the first time.

6. The larvae of house fly, *Musca domestica* (Diptera: Muscidae) were found on the remains stage of decomposed carcass for the first time in Malaysia. There was difference between our observation and other researchers in the occurrence of *Musca domestica* during fresh stage and remains stage in this region. Thus, the occurrence and role of *Musca domestica* on forensic entomological study remain unclear.

7. Ants (Hymenoptera) were reported occurring in all decomposition stages, indicating that ants were not significant indicator for carcasses succession or PMI estimation. However, different species of ants visited the monkey carcasses placed different ecological habitats, indicating that ants can serve as a geographic locality indicator; this information could aid crime scene investigations in the future.

8. More beetles (Coleopteran) species was found in indoor compared to outdoor. Beetles colonize on carcasses solely according to geographical regions and it can serve as an indicator of geographic locality. In this study, the families and species of Coleopteran visiting monkey carcasses in lowland and montane forested areas were identified, and their distribution over the decomposition stages was determined. No beetles were observed to visit carcasses placed in coastal area. Data obtained from this study may help to interpret the Coleopteran evidence in forensic cases occurring in similar ecological habitats. More studies related to beetles succession should be conducted before it can be used as a significant geographical and a PMI indicator.

9. Forensic entomological study conducted on incinerated carcasses emphasizes that:
   (i) Incineration effect does not deter the arrival and oviposition of forensically important flies;
   (ii) Incineration effect does not make any difference on the fauna succession pattern between incinerated and non-incinerated carcasses, with sequence from Calliphoridae, Sarcophagidae and Muscidae; and
   (iii) Decomposition rates on incinerated carcasses were faster than non-incinerated carcasses.

10. Forensic entomological study conducted on carcasses submerged in freshwater river emphasizes that:
   (i) PMI estimation on carcasses submerged in freshwater river was delayed by a minimum period of 2 days;

(ii) There was no difference in the fly succession pattern between carcasses submerged in freshwater river and placed outdoor, with the sequence from Calliphoridae, Sarcophagidae and Muscidae;

(iii) *Chrysomya megacephala* was the dominant adult flies observed to visiting the carcasses, but only *Ch. pinguis* and *Hemipyrelia* spp. were found to be the dominant maggots colonizing on the carcasses; and

(iv) Decomposition rates on carcasses submerged in freshwater river were slower than carcasses placed outdoor.

11. Forensic entomological study conducted on carcasses treated with insecticide concluded that malathion repelled and delayed the insect invasions of the carcasses, especially flies for at least 7 days. After that, although malathion was still present, the concentration might not be high enough to repel and kill the flies to oviposit their eggs on the carcasses.

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