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A preliminary study on the decomposition and dipteran associated with exposed carcasses in an oil palm plantation in Bandar Baharu, Kedah, Malaysia

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Abstract. This study was carried out in an oil palm plantation in Bandar Baharu, Kedah using monkey carcasses and focuses in documenting the decomposition and dipteran colonization sequences in 50 days. This is the first study of Diptera associated with the exploitation of carcasses conducted in the north of peninsular Malaysia during the dry and wet seasons thereat. During the process of decomposition in both seasons, five phases of decay were recognized namely fresh, bloated, active decay, advance decay and dry remain. In this decomposition study, biomass loss of carcass occurred rapidly during the fresh to active decay stage due to the colonization and feeding activity of the Diptera larvae. 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 the wet season. Twenty one species of adult Diptera were identified colonizing carcasses in the study period. Among the flies from the family Calliphoridae, *Chrysomya megacephala* Fabricius and *Chrysomya nigripes* Aubertin were recognized as the earliest arrivals on the first day of exposure. Adult *Ch. nigripes* was abundant for approximately two weeks after placement of the carcasses. By comparing the percentages of adults collected during the study period, the calliphorids abundance in percentages in wet season was 50.83%, but in dry season, the abundance was only about 35.2%. In contrast, the percentage of Sphaeroceridae in wet season was only 3.33%, but in the dry season, the abundance was 20.8%. Dipteran in family Phoridae, Piophilidae, Sepsidae, Drosophilidae and Dolichopodidae colonized the carcasses for a long period of time and were categorized as long term colonizers.

INTRODUCTION

Forensic entomology is the study of insects associated with carrion in order to assist legal investigations and predominantly concern in estimating PMI. Regularly the postmortem interval (PMI) is a primary concern in investigation of homicides and other untimely deaths. Such information can be provided by using carefully documented information on insect development and their succession on dead bodies. Basically, pathologists can estimate the time of death based on several medical parameters (Henssge *et al.*, 1995) but these are only valid for the first few hours after death, becoming less valuable after that and usually not used beyond about 72 hours. Forensic entomology is the most accurate and frequently the primary method in determining time of death when more than a day or two have elapsed (Kashyap & Pillai, 1989). It continues to be valuable up to a year or more after death. Forensic entomology is also useful to determine whether the body has been moved from one site to another or the body has been disturbed after death, the position and presence of wounds and others. The successional pattern of insect colonization on dead body that exists naturally can be applied by an investigator to determine the time of death in a long-term time frame. In the cases of remains that are found within weeks, months and more, forensic

entomologist often need the insect succession data to estimate the PMI (Anderson, 2001). It is natural that the succession of arthropod varies depending on many factors such as the attraction to remains, geographical differences, seasons, weather, sun exposure, urban or rural scenarios, place of body found and the body condition (Anderson, 2001). In peninsular Malaysia, dry and wet periods which are affected by monsoonal wind are familiar. This study introduced a preliminary database of the successional pattern of Diptera in northern side of Malaysia with regard to two distinct pattern of climate, wet and dry. In peninsular Malaysia, between the wet and dry seasons, there is a significant difference especially in the pattern and cumulative rainfall. The species of Diptera associated with carcasses and their pattern of colonization in different weather conditions have not been documented in Malaysia. This study discussed the local species of dipterans and their successions on three replicates of monkey carcasses in an oil palm plantation in Bandar Baharu, Kedah, Malaysia.

MATERIALS AND METHODS

Study site

The study was conducted in an oil palm plantation in Kampung Bukit Tok Din, Sungai Kechil Ulu, 34950 Bandar Baharu, Kedah, peninsular Malaysia (5º8' N, 100º30" E) from 13th April 2004 to 14th October 2004. The site located in a lowland area was occasionally flooded after heavy rain. Other than oil palm trees (*Elaeis guineensis*), the floor of the experimental area are dominated by cogon grass (*Imperata cylindrica*), buffalo grass (*Paspalum conjugatum*), American rope (*Mikania micrantha*), tropical carpet grass (*Axonopus compressus*) and Siam weeds (*Eupatorium odoratum*). The most common animal species encountered in this area are monitor lizard (*Varanus* sp.), long tail macaque (*Macaca fascicularis*), wild boar (*Sus scrofa*), black cobra (*Naja naja*), king cobra (*Ophiophagus hannah*) and rats (*Rattus* spp.). In Malaysia, oil palm plantation expands to 3.37 million hectares and a significant amount of forest is being converted to this plantation due to the high demand for the crop. Records have shown that oil palm plantations are common sites for dead bodies.

Experimental animal model

To conduct the study, mature long tail macaques (*Ma. fascicularis*) between 2.45 to 5.3 kg (mean 3.73 kg) were chosen as the animal model due to their availability in this habitat. This species is a pest in the farm, abundant in oil palm plantations and frequently killed by farmers to protect their crop as the Wild Life Department of Malaysia permits farmers to kill the animal to control their population or to minimize damages in the plantations and orchards. In the plantation the monkeys were killed by a farmer who used a single shot from a one barrel shot gun and collected shortly after they were shot. The dead monkeys therefore exhibited gunshot trauma produced by 12 gauge shotgun ammunition and they therefore provided an experimental model of decay following gunshot trauma.

On site and laboratory procedure

In Malaysia, rainfall is affected by the North-East and South-West monsoons which bring heavy rainfall. Based on 33 years of Malaysian weather history from 1951 to 1983, an obvious dry and wet season is experienced in north peninsular Malaysia due to these monsoonal winds. The selected time of the study period was based on this report and additional past year's cumulative rainfall records provided by Malaysia Meteorological Department (MMD). Thus, the study was conducted in 13th April to 1st June for the dry season while the time period from 16th August to 14th October was selected as the wet season. In every season three replicate monkey carcasses were used. The carcasses were left exposed on the ground, protected by wire mesh cage to prevent them being dismembered and/or removed by large scavengers such as dogs and wild boars and placed 40m apart. Each carcass was placed between two mature trees to standardize microclimate. Overall, six carcasses were

used in the study. To lift each carcass for weighing purpose, a platform build by wire mesh was placed under the carcasses. Weighing was done with extreme care to minimize the effect of lifting on disarticulation of bone and the insect succession. Weighting was done only once per day using spring scale weight (Super Samson, Salter).

All the carcasses were visited at 10 am everyday. Adult flies were collected using a hand net whilst their larvae were collected using forceps. At the same time the carcasses were weighed and pup tent fly traps were placed. All pup tent fly trap were collected after 2 hour of placement, at 12pm everyday. Diptera especially blowflies (Diptera: Calliphoridae) frequenting the carcasses were captured using an insect net and pup tent fly trap (Haskell & Williams, 2000) from fresh stage to until no more flies were observed. Pup tent fly traps were covered by plastic roof to prevent the trap against rainfall. The temperature and humidity were recorded using thermohygrograph which was placed beside the carcass placement site. The rainfall data were obtained from the nearest weather station, Parit Buntar Hospital, Perak which is about 5 kilometer from the study site. Samples collected in the field were identified to the lowest taxonomic rank to which they could confidently be assigned. The flies collected were identified using external morphological characters and identification keys in Kurahashi *et al.* (1997), Oosterbroek (1998), Castner & Byrd (2000), Greenberg & Kunich (2002), Smith (1986), Baharudin (2002), Kurahashi (2002) and Couri (2004).

RESULTS

Weather and environmental condition at the study site

The total amount of rainfall during the two months sampling period in dry season was 192.6 mm with 19 rainy days and the total amount of rainfall in wet season period was 619.2 mm with 29 rainy days (Figures 1 and 2). In the dry season, rainy days were recorded occasionally between day 1 and day 30 but effectively stopped thereafter (Figure 1). By contrast, in the wet season, rainy days occurred throughout the study period (Figure 2). In the dry season, the lowest temperature recorded was 22.2ºC at day 26 and the highest temperature was 35.7ºC at day 51 (Figure 1). On the other hand, in the wet season, the lowest temperature recorded was 21.3ºC at day 9 and the highest temperature was 35.4ºC at day 6 (Figure 2).

Carcasses decomposition

Fresh stage: Dry season (day 1-2)
Wet season (day 1-2)
Adult Calliphoridae were observed feeding on exudates around the eyes, nose, mouth and wounds of the monkey corpses within 5 minutes of them being exposed and blowfly eggs were laid at the eyes and mouth during the course of the first day. By the end of the fresh stage on day 2 the carcasses were infested with first instar larvae of calliphorid flies although there were only small numbers of these.

Bloat stage: Dry season (day 2-4)
Wet season (day 2-4)
The physical signs of the bloated stage were accompanied by the release of a powerful smell of decomposition. Green skin staining due to internal autolytic and bacterial breakdown of hemoglobin appeared in all carcasses. During this period, most of the eggs laid by gravid female Calliphoridae hatched and first and second instar larvae were abundant at the mouth, nose and eyes. In addition, second and third instar larvae of Sarcophagidae were found in the mouth and in the throat.

Active decay stage: Dry season (day 4-8)
Wet season (day 4-7)
Penetration of the abdominal wall and subsequent deflation of the abdominal wall mark the start of the active decay stage. Odor of decomposition had increased at this time. This stage started on day four in both seasons (Table 1). The body hairs were lost during this period and the skin became black. There was a rapid decrease of weight leaving approximately only 20%–30% of total body

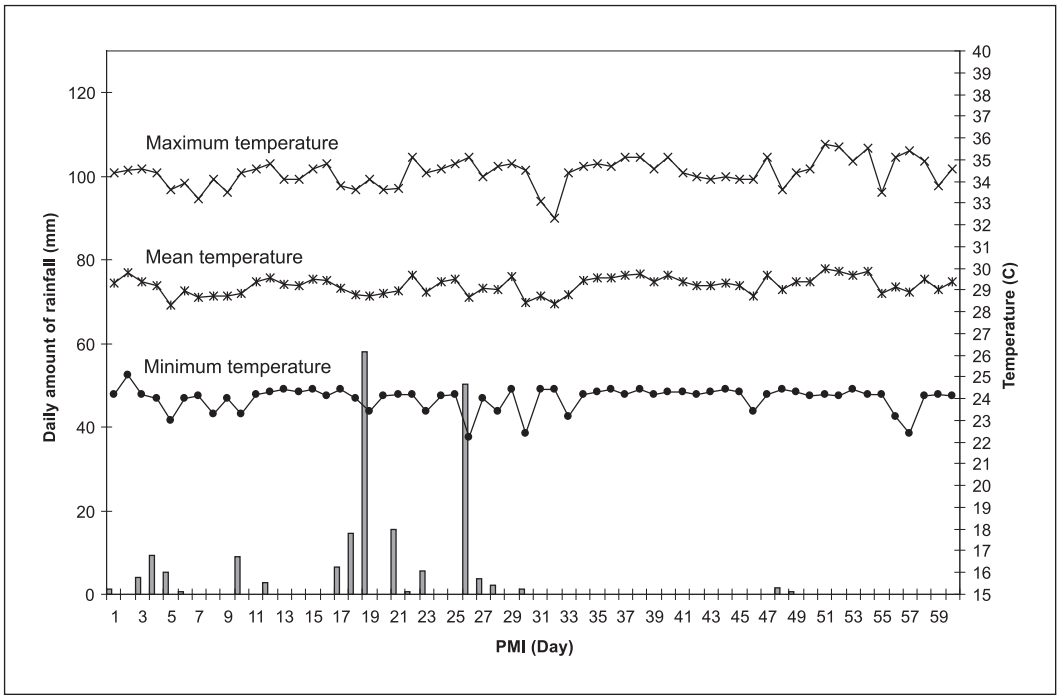

Figure 1. Daily amount of rainfall and environmental ambient temperatures during the dry season.

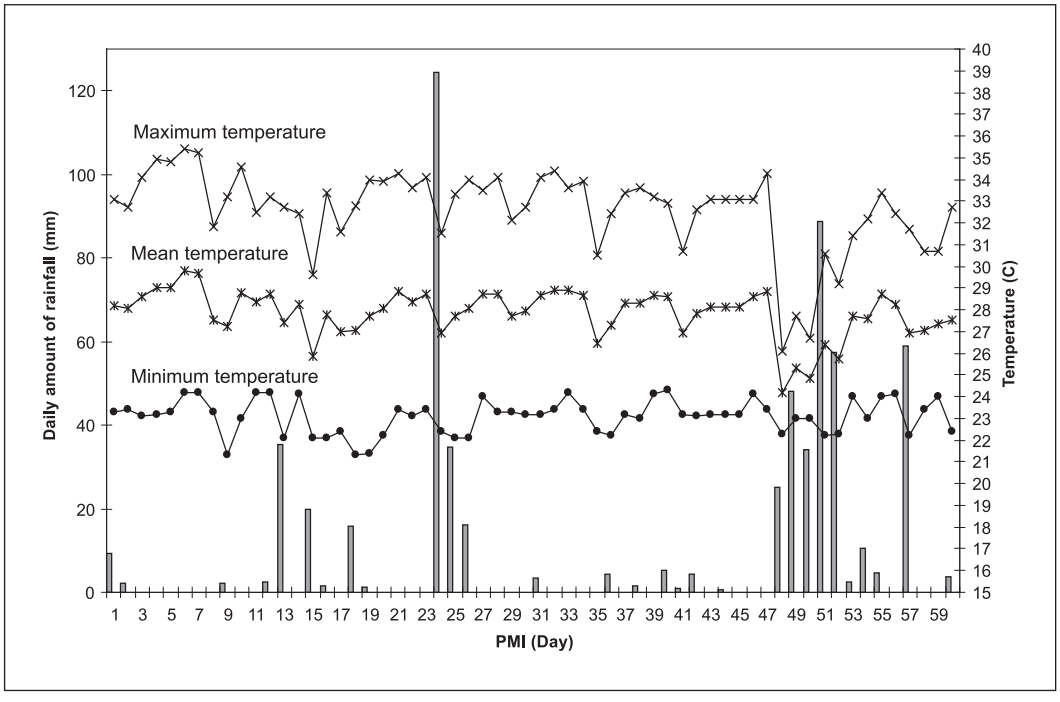

Figure 2. Daily amount of rainfall and the environmental ambient temperatures during the wet season.

Table 1. Estimated time range for each carcass decomposition stage in dry and wet seasons

Decomposition stage	Dry season (day)	Wet season (day)
Fresh	1–2	1–2
Bloated	2–4	2–4
Active decay	4–8	4–7
Advance decay	8–23	7–14
Dry remain*	23–not recorded	14–not recorded

*the dry remain stage for both seasons extended beyond the end of the study period.

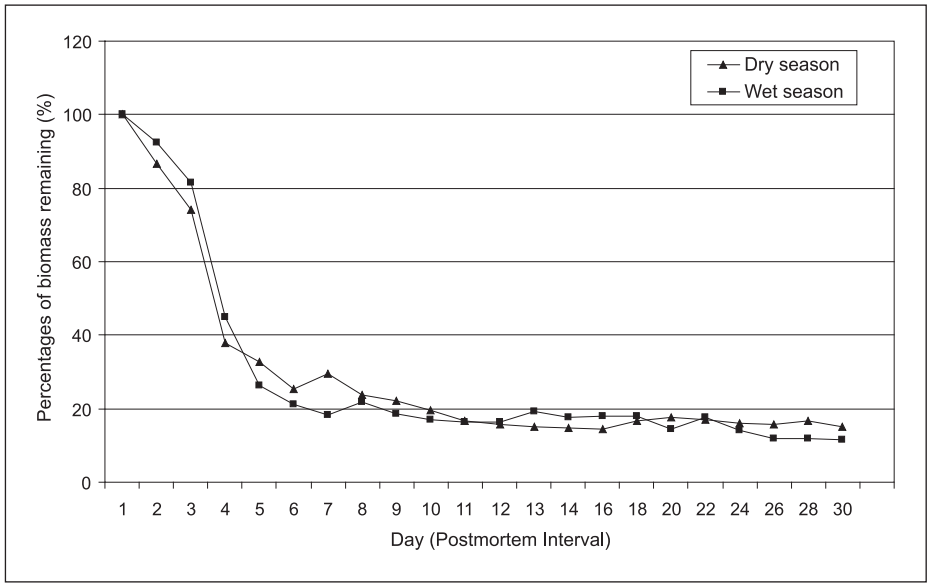

Figure 3. Mean percentages of biomass remaining of carcasses (n=3) during dry and wet season.

weight (Figure 3). The large mass of third instar larvae of *Chrysomya rufifacies* formed within the collapsed abdomen, and also at the genitalia, mouth, nose and eyes. At the end of active decay stage, flesh was removed leaving only the skin and the abundance of adult Calliphoridae was reduced by this time.

Advance decay stage: Dry season (day 8-23)
Wet season (day 7-14)
In the advance decay stage, most of the post feeding larvae left the carcasses. Only bones, cartilage, hair and small portions of tissue remained. Biomass loss slowed down at this time. The carcasses had started to dry and a cheesy odor developed. Many intact pupae of *Ch. rufifacies* were scattered on and underneath the carcasses.

Dry remain: Dry season (day 23)
Wet season (day 14)
This was characterized by the bodies being reduced to bones with little cartilage remaining. In most carcasses only disarticulated bones and dry skin remained. A few postfeeding Calliphoridae larvae were observed during this time. There were many

empty pupal cases scattered around and under the carcasses. At this time most of the maggots had pupated and odor of carcass disappeared. In dry season, dry remains were first observed on day 23 while in wet season this stage was reached by day 14 (Table 1). The dry remain stage did not end in both seasons even after the study period ended on day 50.

Species composition

In this study, a total of 4239 individuals of adult Diptera frequenting monkey carcasses were collected on the pup tent fly trap during the 50 days period. Twenty one species of adult Diptera belonging to eleven families were identified. In the dry season a mean of 713(n=3; range 590-888) individual per carcass were collected while in the wet seasons a mean of 700(n=3; range 645-713) individual per carcass were collected. This indicates that there was no difference in the numbers of dipterans collected per carcass between seasons although there were differences in the composition of Diptera by family (Figure 4). Adult Calliphoridae comprised 50.83% of all adult Diptera collected during the wet season but only 35.2% in dry season (Figure 4). In contrast, the Sphaeroceridae family was abundant in the dry season with 20.8%, but only 3.33% in the wet season (Figure 4). Among other families, no large differences were recognized between seasons.

Among the Calliphoridae, *Chrysomya megacephala* and *Chrysomya nigripes* were the first flies collected on carcasses in both seasons although adult of *Ch. nigripes* was the predominant species. Several species from other families were also recorded frequenting the carcasses on the first day. These species included sarcophagids, *Musca sorbens* Weidemann, *Megaselia* sp., *Piophila* sp., *Leptocera* sp., *Drosophila* sp. and *Psycoda* sp in the dry season while *Hydrotaea* sp., *Megaselia* sp. and *Psychoda* sp. were recorded in the wet season (Figure 5 and 6). In the successional pattern in dry season, *Megaselia* sp. and *Leptocera* sp. showed a long-term consistent colonization from day one until 44 (Figure 5). This is the longest consistent colonization period in the present study. In dry and wet seasons, the first appearance of black soldier fly, *Hermetia illucens* Linnaeus was recorded on day two and six of carcasses placement respectively (Figures 5 and 6).

DISCUSSION

We initially expected that during the wet season there would be an increase in the Diptera numbers due to the availability of moist flesh as an oviposition medium, as reported by Ashworth & Wall (1994). In contrast, this study indicated that high frequencies of rainfall during wet seasons (29 days) might have disturbed flight activities thereby decreasing the number of some Diptera species visiting the carcasses. However, this might be limited to the smaller flies. This was evident when we collected only 3.33% of Sphaeroceridae in wet season instead 20.8% in dry seasons. This pattern was similar with the other small flies, i.e. the Sepsidae, Phoridae, Psycodidae families. In contrast, the larger flies, (Calliphoridae and Muscidae) were collected in greater frequencies in the wet seasons than the dry season. The presence of adult Diptera frequenting carcasses in both seasons does not necessarily mean that oviposition occurred because some species visit carcasses in search of food or a mate.

In the wet season we recorded that carcasses needed less time to reach the dry remains stage of decomposition. According to Archer (2003), high amount of rainfall can reduce the length of decay stage by increasing mass loss. We also suggest that for the tropical region, in order to investigate the decomposition of carcass or remains, the daily pattern of rainfall in the first two weeks plays a major role rather than monthly cumulative rainfall. The daily pattern of rainfall in the first two weeks can play a significant role to produce humid environment which helps to keep the tissues moist. High relative humidity can make tissues a suitable environment for the activity of bacteria. Thus the amount of rainfall in the first two weeks of decomposition is more important than

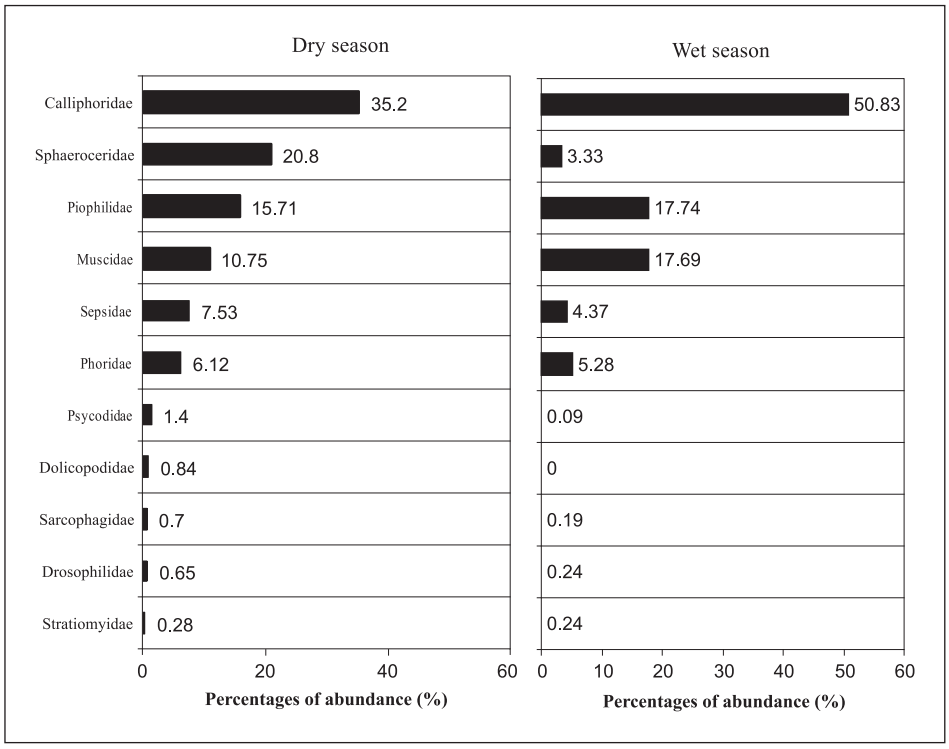

Figure 4. Abundance plot (percentages) of adult flies (Diptera) by family on carcasses in dry and wet seasons.

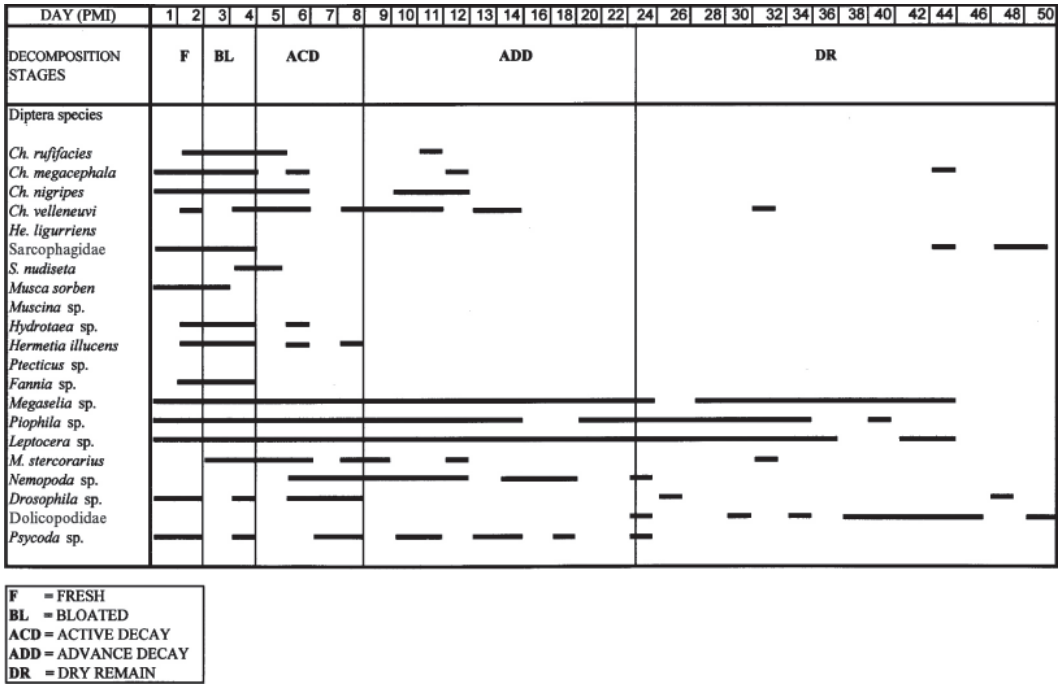

Figure 5. Adult Diptera succession on monkey carcasses in five decomposition stages during dry season.

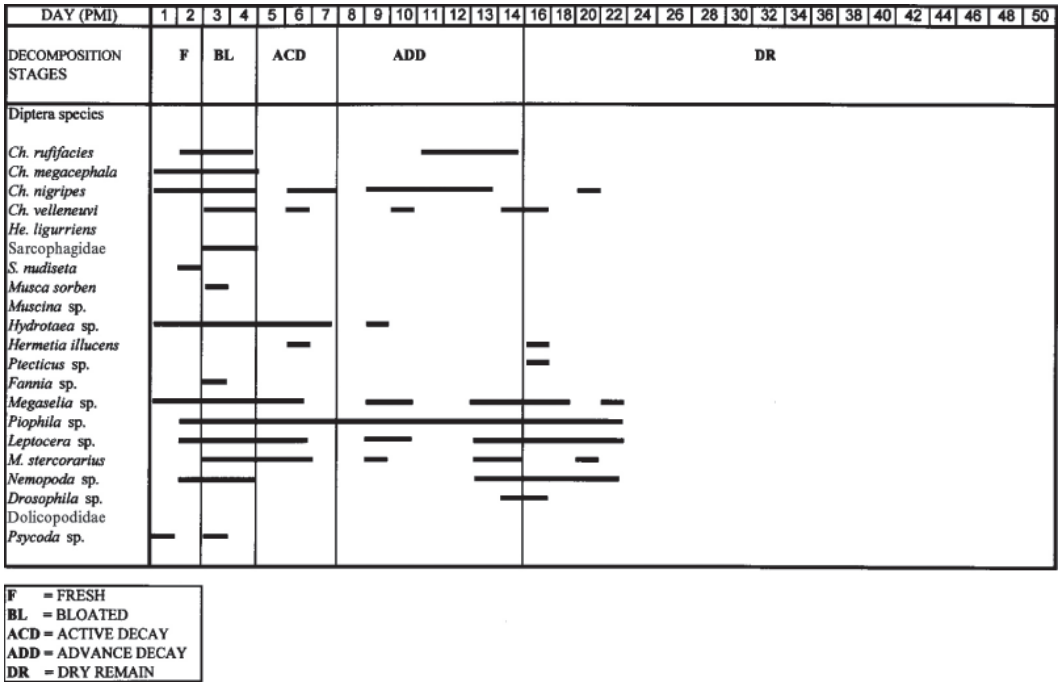

Figure 6. Adult Diptera succession on monkey carcasses in five decomposition stages during wet season.

monthly cumulative rainfall in studying the decomposition in tropical climate and also for PMI estimation.

The blowflies (Calliphoridae) species collected during the present study are among the known common blowflies that can be found in Malaysia and Singapore (Kurahashi *et al.*, 1997). Some of them, primarily *Ch. rufifacies* and *Ch. megacephala* were frequently encountered on dead body in previous case studies in Malaysia (Nor Afandy *et al.*, 2001; Noratiny *et al.*, 2002). According to Goff (2000), the occurrence of these blowflies on a dead body is valuable in accurately estimating a postmortem interval during the two weeks time frame after death.

In this study, among the Calliphoridae, the adult of *Ch. nigripes* and *Ch. megacephala* were the earliest species collected on the carcasses in both seasons. However another blowfly, *Ch. rufifacies* was recorded to arrive later, on the second day in both seasons. In Hawaii, several studies indicated that *Ch. rufifacies* arrived at carrion in the first day of placement of pig carcasses (Early & Goff, 1986; Tullis & Goff, 1987), which differed from this study. This difference is possibly caused by the predacious ability of *Ch. rufifacies* on carcasses. Only after the presence of *Ch. megacephala* eggs or larvae, adults of *Ch. rufifacies* may be stimulated to oviposit their eggs with the cluster of *Ch. megachepala* eggs or larvae, so then the larvae of *Ch. megacephala* can be consumed by the larvae of *Ch. rufifacies* as an additional food source. This might be the primary reason why the colonization of adult *Ch. rufifacies* was later than *Ch. megacephala.* In this sudy, *Leptocera* sp. was found on the carcasses from the early until the later stage of decomposition. According to Martinez *et al.* (2006) this species is present in the dry remains stage and was classified as indicator for the dry remains stage.

Throughout the experimental period, adults of other Calliphoridae, *Chrysomya villeneuvi* (Patton) and *Hemipyrellia ligurriens* (Wiedemann) were never found to colonize the carcasses as early as *Ch. megacephala* and *Ch. nigripes.* This may be

due to the intense competition in the blowflies' population. Similar competition also existed in some Muscidae species, where their competition with Calliphoridae and Sarcophagidae reduced the number of this muscid species (Bharti & Singh, 2003). In a previous study, Denno & Cothran (1976) also reported that the competition that exists between adult Calliphorids and Sarcophagids can affect the population size of Sarcophagids.

In the wet season adult *Her. illucens* was recorded on day six of carcasses placement. This finding is similar to that of Tomberline *et al*. (2005) but contrary to Lord *et al.* (1994) who indicated that this species did not colonise dead bodies until 20 to 30 days of the postmortem interval had elapsed. This species has a significant importance in forensic investigation in late postmortem interval and associated with human remains during the advance to dry stage (Lord *et al.* 1994; Nor Afandi *et al.*, 2001)

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