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SOCIAL SCIENCES

Foreword

This is the first time that ESTEEM Academic Journal UiTM Pulau Pinang has come up with 2 publications in a year! Previously, ESTEEM was published once biennially.

For these publications to materialise, I would like to thank Associate Professor Mohd Zaki Abdullah, the Director of UiTM Pulau Pinang for his unflinching support and who always told me, "Go ahead, don't worry about the money!".

Both the Associate Professor Mohd Zaki Abdullah and Dr. Mohamad Abdullah Hemdi, the Deputy Director of Academic Affairs really provided me with a great deal of assistance in ensuring that there are sufficient articles for publishing. Both of them have emphasized the need for lecturers to embark on journal writing. Incidentally this is one of the prerequisites for promotion among the academic staff members of UiTM Pulau Pinang.

I do not think I can run the show alone without the help from the editorial board, reviewers and the cooperation from University Publication Centre (UPENA) of UiTM Malaysia. My special thanks to Mr. Mohd Aminudin Murad for his efficiency in editing articles and to Dr. Khairil Iskandar Othman for speeding up the final stage of printing process.

Since writing is an important criterion in rating a university, I feel it is a great responsibility for me to produce a good journal. Fellow colleagues, let's work closely to put UiTM Pulau Pinang in the final list of Anugerah Kualiti Naib Canselor (AKNC) and Anugerah Kualiti Perdana Menteri (AKPM) by submitting more quality articles to ESTEEM!

Lastly, let me end by thanking all of you for giving your unwavering support to UPENA.

The Chief Editor
November, 2008

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Decomposition and Dipteran Composition on Exposed Carcasses in an Oil Palm Plantation: A Forensic Entomology Study

Azwandi Ahmad
Abu Hassan Ahmad

ABSTRACT

This is the first study on Diptera associated with carcasses carried out in north peninsular of Malaysia with reference to dry and wet climate in Malaysia. During the process of decomposition in both seasons, five phases of decay were identified namely fresh, bloated, active decay, advance decay and dry remain. In this decomposition study, biomass loss of carcass occurred rapidly from the fresh stage to the active decay stage, which was about 50% of body weight removed due to the significant colonization and feeding activity of the Diptera larvae ($p < .05$). Twenty-one species of adult Diptera were identified colonizing carcasses in the study period. In this study, the flies from the family of Calliphoridae, Chrysomya megacephala Fabricius *(Diptera: Calliphoridae) and Chrysomya nigripes Aubertin (Diptera: Calliphoridae) were recognized as the earliest necrophagous insects arrived on the first day of exposure. Adult of Chrysomya nigripes was predominantly frequenting carcasses and abundant in the period approximately of two weeks after placement of the carcasses. By comparing the percentages of adult Diptera collected during the study period, Calliphorids abundance in wet season were 50.83%, but in dry season, the percentage of abundance was only about 35.2%. In contrast, the percentage of Sphaeroceridae family in wet season was only 3.33%, but in the dry season, the abundance percentage was 20.8%. Dipteran in family Phoridae, Piophilidae, Sepsidae, Drosophildae, and Dolicopodidae were colonized carcasses in a long period of time and were categorized as long term colonizers.*

Keywords: *Forensic entomology, decomposition, carcass, oil palm plantation*

Introduction

Forensic entomology is a study of insects and arthropods associated with carrion in order to assist legal investigations and predominantly concern in estimating time since death (also known as Postmortem Interval). Basically, pathologists can estimate the time of death based on several medical parameters (Henssge, Madea, Knight, Nokes, & Krompecher, 1995), but these are only valid for the first few hours after death and become less valuable after about 72 hours. Forensic entomology is the most accurate and frequently the primary method in determining time of death beyond this period and continues to be valuable up to a year or more after death. The development of Diptera larvae and the succession of insect's species can give the estimation of the time of death. The successional pattern of insect colonization on dead body that exists naturally can be applied by investigator to determine the time of death in a long-term time frame. In the cases of remains that were found in weeks, months and more, forensic entomologist often need the insect succession data to estimate the postmortem interval (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 of rural scenarios, place of body found, and the body condition (Anderson, 2001). In peninsular Malaysia, dry and wet season, which 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 patterns of climate, wet and dry. In Peninsular Malaysia, between the wet and dry seasons, it has a significant difference of weather condition especially in the pattern and cumulative rainfall. The species of Diptera associated with carcasses and their pattern of colonization in a different weather condition are not yet documented in Malaysia. Most important, this study discussed the local species dipterans and their successions on three replicate of carcasses in the region, which was located in an oil palm plantation in Bandar Baharu, Kedah, Malaysia.

Materials and Methods

Study Site

The study was conducted in an oil palm plantation at Kampung Bukit Tok Din, Sungai Kechil Ulu, 34950 Bandar Baharu, Kedah, northern of Peninsular Malaysia (5º 8" N, 100º 30" E) from April 13, 2004 to October 14, 2004. The site is located in a lowland area and 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 iguana (*Varanus bengalensis)*, long tail macaque (*Macaca fascicularis*), wild boar (*Sus scrofa*), black cobra (*Naja leucodira*), king cobra (*Ophiophagus hannah*), and rats (*Rattus* sp.). 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. Due to the large area of this plantation, it is one of the most frequent areas where the dead bodies were found and the probability to find decomposed dead bodies in such place in the future is high.

Experimental Animal Model

To conduct the study, matured long tail macaques (*Macaca 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, which is abundant in oil palm plantation. They are frequently killed by farmers to protect their crops as the Wild Life Department of Malaysia have permitted farmers to kill them to control their population or to minimize damages in the plantations and orchards. In the plantation the monkeys were eliminated by farmers using single shot with one barrel shot gun. As experimental model, these animals were collected in the oil palm plantation slightly after they were eliminated by farmers. In overall, the dead monkeys present gunshot trauma produced by 12 gage shotgun ammo and this will mimic the dead body, which decomposed with the presence of gunshot wound.

On Site and Laboratory Procedure

In Malaysia, rainfall is affected by the North-East and South-West monsoons, which bring heavy rainfall. For the months during April–May and September–October, less rain is found because of changes in monsoonal winds. The selected time period of the study was regarding to this monsoonal winds and the cumulative rainfall for the past years. Thus, the study was conducted from April, 13 to June, 1 during the dry season, while August, 16 to October, 14 was selected, as it was during the wet season. In every season, three replicate of monkey carcasses were used. The carcasses were left exposed on the ground, protected by wire mesh cage to prevent scavengers, and placed 40 m between each other. Each carcass was placed between two mature trees to standardize microclimate. As overall, six carcasses were used. For weighing purpose, wire mesh platform was prepared under the carcasses. All carcasses were visited at 10:00 a.m. everyday. Observation, collecting adult flies using hand net, larvae collecting using forceps, carcasses weighing, and placement of four pup tent fly traps were done at this time. All pup tent fly traps were collected after 2 hours of placement, at 12:00 p.m. everyday. Diptera especially blowflies (Diptera: Calliphoridae) frequenting on the carcasses were captured using insect net and pup tent fly trap (Haskell & Williams, 2000) from fresh stage until no more flies were observed. Pup tent fly traps were covered with plastic roof to prevent the traps against rainfall. In order to study the relative abundance of dipteran larvae, a 15 ml measuring scoop was used to collect the larvae from three areas of the carcass (mouth, stomach, and genitalia); one scoop of larvae was collected from each area. The abundance of larvae in each scoop from those three areas were combined, so the total of 45 ml volume of scoop was used to measure the larvae abundance on the carcasses. The temperature and humidity were recorded using thermohygrograph, which was placed beside the carcass placement site. The rainfall data was obtained from the nearest weather station, Parit Buntar Hospital, Perak, which is about 5 km 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 with referring to the identification key developed by Kurahashi Benjaphong, and Baharudin (1997).

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 during two months sampling in wet season period was 619.2 mm with 29 rainy days (Figures 1 and 2). As what has been expected, the difference between wet and dry seasons is recognizable. In dry season, rainy days occurred between the first day after carcass placement and stop after 30 days after carcass placement (Figure 1). In wet season, the rainy days occurred throughout the study period (Figure 2). Small differences of temperature were recorded between dry and wet season, which dry and wet season showed the temperature between 20ºC to 32ºC and 19ºC to 35ºC, respectively.

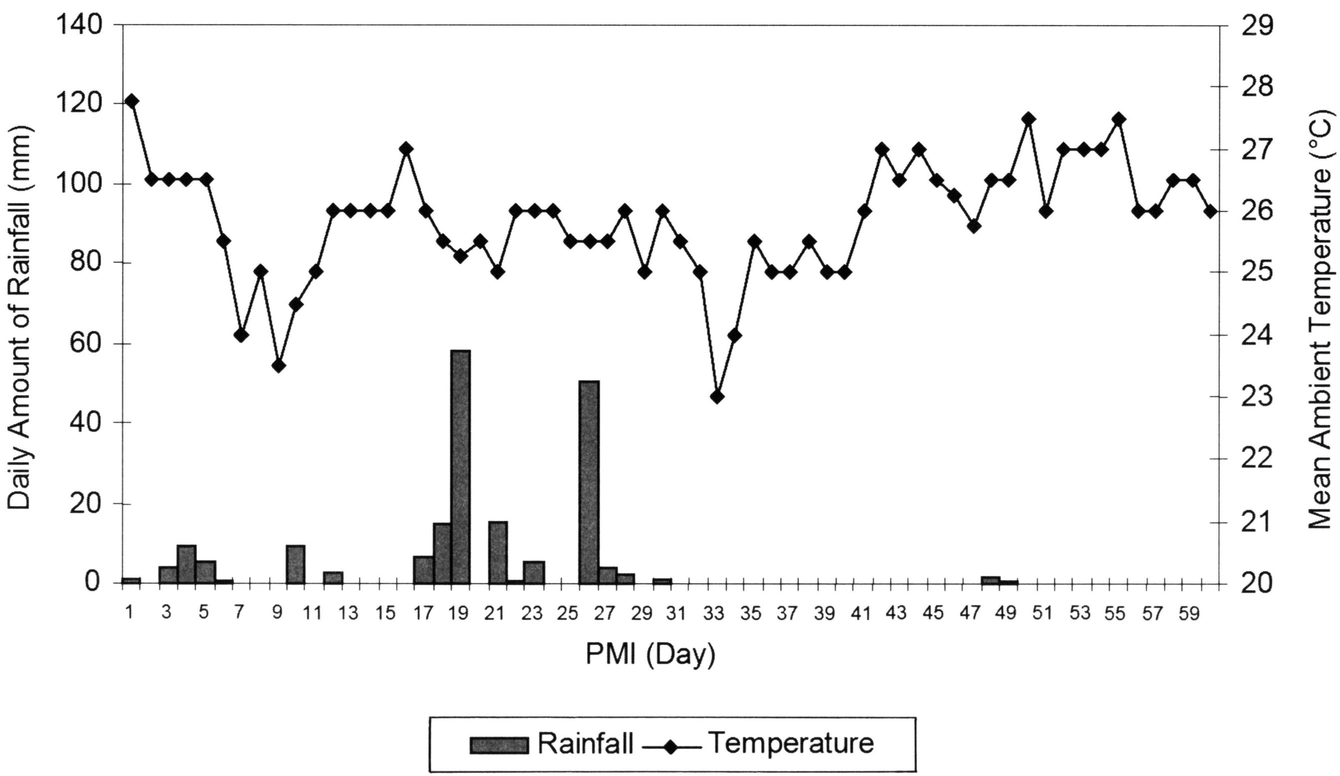


Figure 1: Daily Amount of Rainfall and Mean Ambient Temperature During the Dry Season. PMI = Postmortem Interval

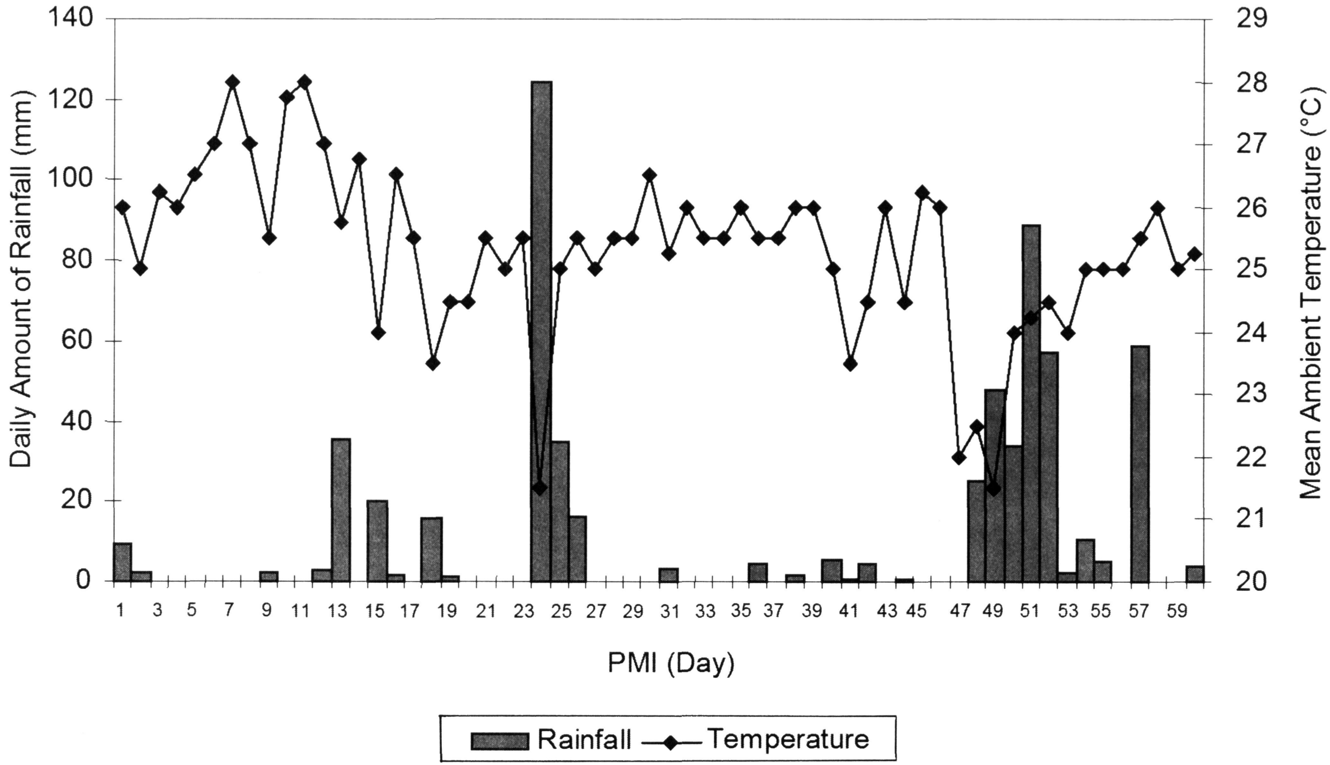


Figure 2: Daily Amount of Rainfall and Mean Ambient Temperature During the Wet Season. PMI = Postmortem Interval

Carcasses Decomposition

Fresh Stage (Figure 3)

Adult Calliphoridae were observed on the carcasses within 5 minutes after exposure. These adults began feeding on exudates around the eyes, nose, mouth, and wounds while in search of suitable oviposition sites. There was a slight increase in carcasses total body weight due to the absorption of moisture from rainfall; however, this increase was not noticeable. On the first day, the eggs were firstly observed laid at the eyes and mouth. By the end of the fresh stage on day 2, the carcasses were infested by the early immature Diptera larvae but in a very small numbers. They are consisting of the first instar larvae of the calliphorids.

Bloated Stage (Figure 4)

The bloated stage was evident when odor had developed and recognized as the original phase of decomposition. Most of the eggs laid by gravid female Calliphoridae hatched while the first and second instar larvae were abundant at the mouth, nose, and eyes at this time. During the bloated stage the second and third instar larvae of Sarcophagidae were found in the mouth and in the throat. Green skin staining due to internal bacterial breakdown of hemoglobin appeared in all carcasses.

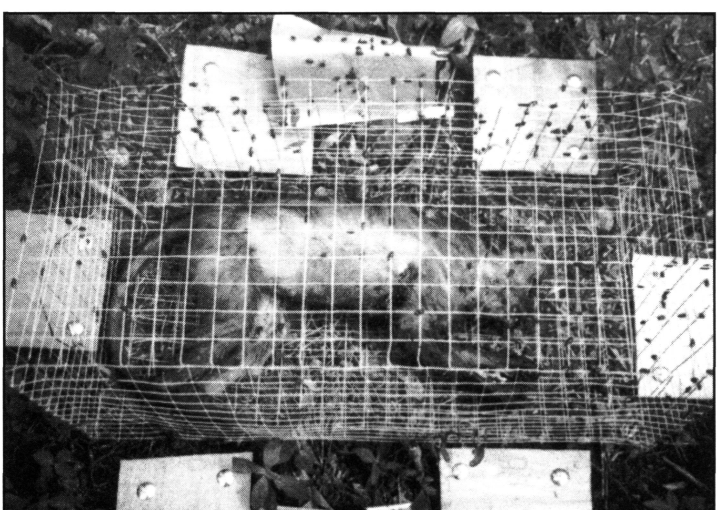


Figure 3: Fresh Stage

Figure 4: Bloated Stage

Active Decay Stage (Figure 5)

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 (see Table 1). The hairs were completely removed and the skin became black. In those carcasses, a rapid decrease of weight occurred leaving approximately only 20% of total body weight (see Figures 8 and 9). A large mass of third instar larvae of *Chrysomya rufifacies* (Macquart) were present at the collapsed abdomen, genitalia, mouth, nose, and eye. In order to understand the relationship of the abundance of larvae and rapid decrease of body weight, a correlation analysis was made to correlate the larvae abundance with the biomass remaining in the sequence of 22 days. There were significant correlations of larvae abundance ($p < .05$) with biomass remaining, which the correlation coefficient (r) of .709 and .799 in the dry and wet seasons, respectively (Figures 8 and 9). Figures 3 and 4 also indicated that the abundance of Diptera larvae was recorded high at the sharpest slope of biomass remaining plot that reflect the active feeding activity against carcasses tissue. At the end of active decay stage, flesh was removed and left with the skin. The abundance of adult Calliphoridae was reduced by this time.

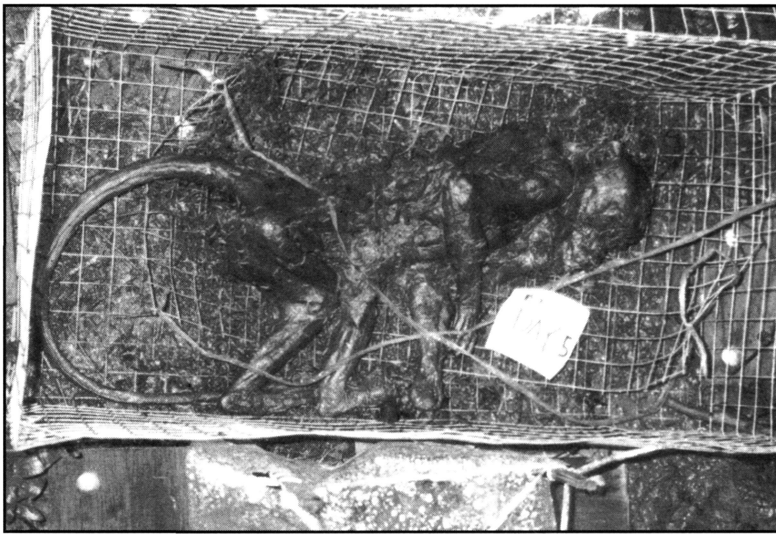


Figure 5: Active Decay Stage

Table 1: Estimated Time Range for Each Carcass Decomposition Stage in Dry and Wet Season

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

Note: * The dry remain stage did not end even the study period had terminated

Advance Decay Stage (Figure 6)

In the advance decay stage, most of the post feeding larvae left the carcasses. Only bones, cartilage, hair, and small portions of tissue were remained. Biomass lost was slow down at this time. The carcasses had started to dry and a cheesy odor was developed. Many intact pupae of *Ch. rufifacies* were scattered on and underneath the carcasses.

Dry Remain (Figure 7)

This is characterized by bones with little cartilage remaining. In most carcasses only disarticulated bones, dry skin, and hair were remained. A very few numbers of postfeeding Calliphoridae larvae were observed during this time. There were many empty pupa cases scattered around and under the carcasses. At this time most of the pupae hatched and odor of carcass disappeared. In dry season, dry remain started on day 23 while in wet season it started on day 14 (see Table 1). The dry remain stage was very long and this stage did not end although the study period was terminated.

Figure 6: Advance Decay Stage

Figure 7: Dry Remain Stage

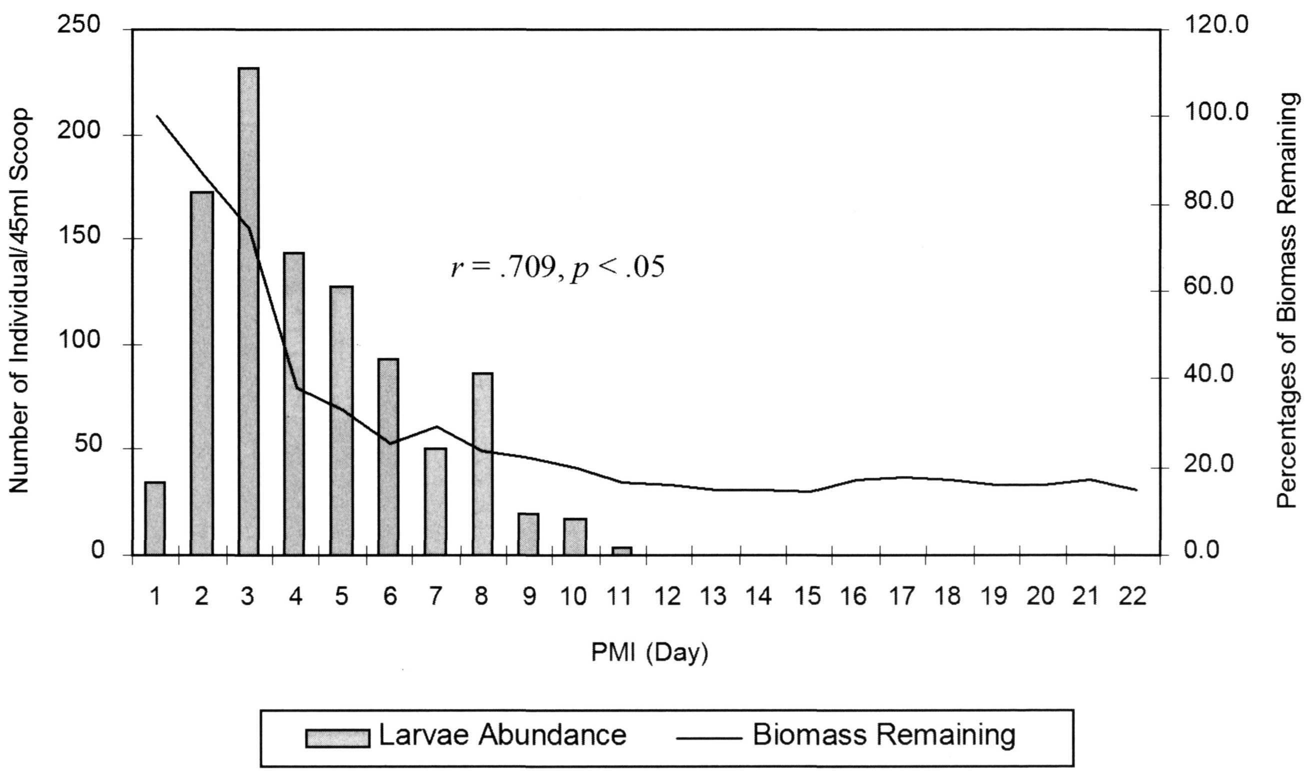


Figure 8: The Relationship of Larvae Abundance versus Biomass Remaining (%) of Carcasses ($n = 3$) during Dry Season.
PMI: Postmortem Interval

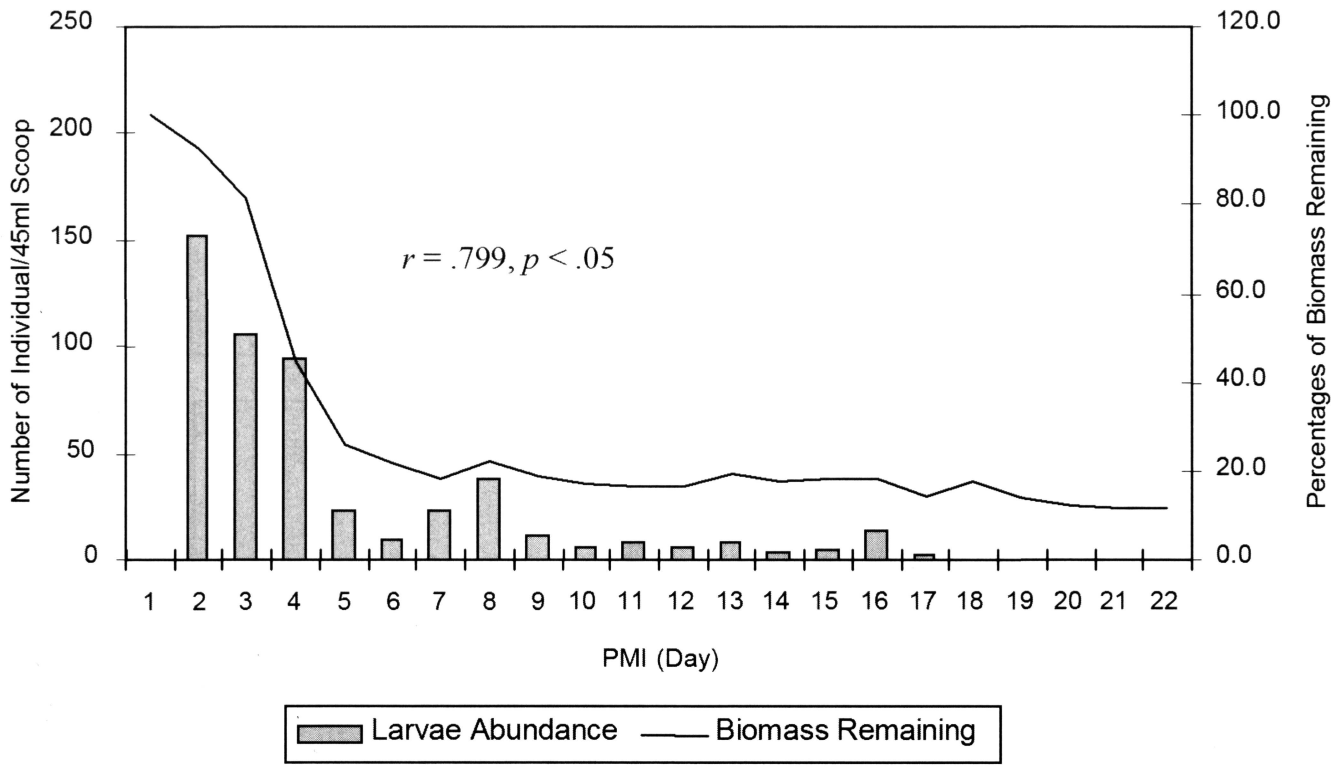


Figure 9: The Relationship of Larvae Abundance versus Biomass Remaining (%) of Carcasses ($n = 3$) during Wet Season.
PMI: Postmortem Interval

Species Composition

In this study, a total of 4,239 individuals of adult Diptera frequenting monkey carcasses were collected with the pup tent fly traps in 50 days period. Twenty one species of adult Diptera belonging to eleven families were identified. In dry season, a mean of 713 (n = 3) individuals per carcass were collected while in wet season a mean of 700 (n = 3) individuals per carcass were collected. This shows a small difference of the number of diptera collected per carcass between two seasons, but the composition of Diptera by family in both seasons shows differences (see Figure 10). By comparing the percentages of Diptera collected, adult Calliphoridae collected in the wet season was 50.83%. However only 35.2% of adult Calliphorids were collected in dry season (Figure 10). In contrast, the Sphaeroceridae family was abundant in the dry season with 20.8%, but only 3.33% found in the wet seasons (Figure 10). By comparing the composition of adults Calliphoridae with Sphaeroceridae during both seasons, it shows a different pattern. Among other families, no large differences were recognized between the two seasons.

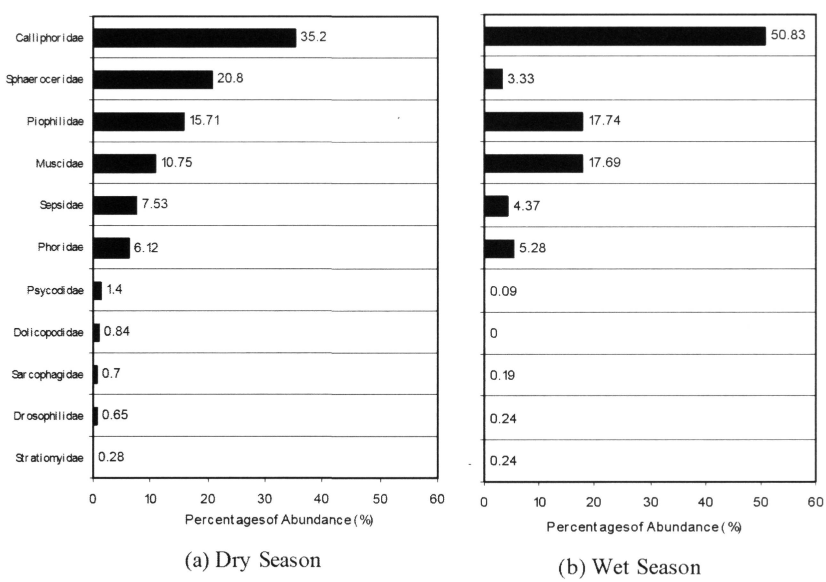
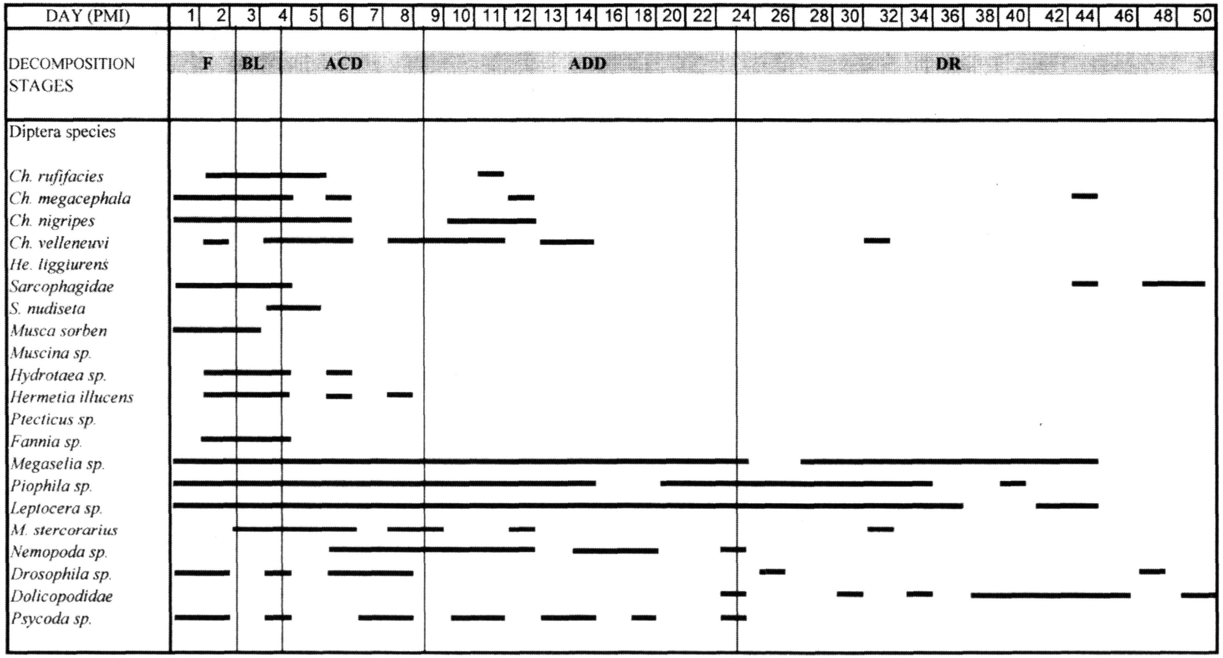


Figure 10: Abundance Plot (Percentages) of Adult Flies (Diptera) by Family on Carcasses in Dry and Wet Season

In this study adult of *Ch. nigripes* was the predominant species collected from the carcasses. On the carcasses in both seasons, the earliest colonizers were the species of *Ch. megacephala* and *Ch. nigripes*. In dry season, adults of Calliphoridae colonized carcass about six days after placement and left carcass for several days after that (see Figure 11). This family resurfaced as early as day eight. In wet season, the colonization of calliphorids stopped on day five and resumed as early as a day after (see Figure 12). Among the Calliphoridae, *Ch. megacephala* and *Ch. nigripes* were the first flies collected on carcasses in both seasons. Although the Calliphoridae got a special attention being the first flies colonizing carcasses, several species from other families were also recorded frequenting carcass on the first day. The species were Sarcophagid, *Musca sorbens* Weidemann, *Megaselia* sp., *Piophila* sp., *Leptocera* sp., *Drosophila* sp., and *Psycoda* sp. (see Figure 11) in the dry season while *Hydrotaea* sp., *Megaselia* sp., and *Psychoda* sp. in the wet season. In the successional pattern in dry season, *Megaselia* sp. and *Leptocera* sp. showed a long-term consistent colonization from day one until day 44 (see Figure 11). This is the longest consistent colonization period in the present study. In dry and wet season, the first appearance of black soldier fly, *Hermetia illucens* Linnaeus, was recorded on day two and day six of carcasses placement respectively (see Figures 11 and 12).

Discussions

In the early hypothesis, we expected that wet season will increase the Diptera numbers due to the availability of moist flesh as an oviposition medium, as reported by Ashworth & Wall (1994). In contrast, the result in this study shows that too high frequency of rainfall during wet season (29 days) might have disturbed the flying activities of some Diptera family thus decreased the number of the particular Diptera visiting 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 season. This pattern was resembled with the Sepsidae, Phoridae, and Psycodidae family. In contrast, the larger flies, Calliphoridae and Muscidae, were collected in greater frequencies in wet season 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 carcass in searching for food or mating.



F = FRESH
BL = BLOATED
ACD = ACTIVE DECAY
ADD = ADVANCE DECAY
DR = DRY REMAIN

Figure 11: Adult Diptera Succession on Monkey Carcasses in Five Decomposition Stages During Dry Season

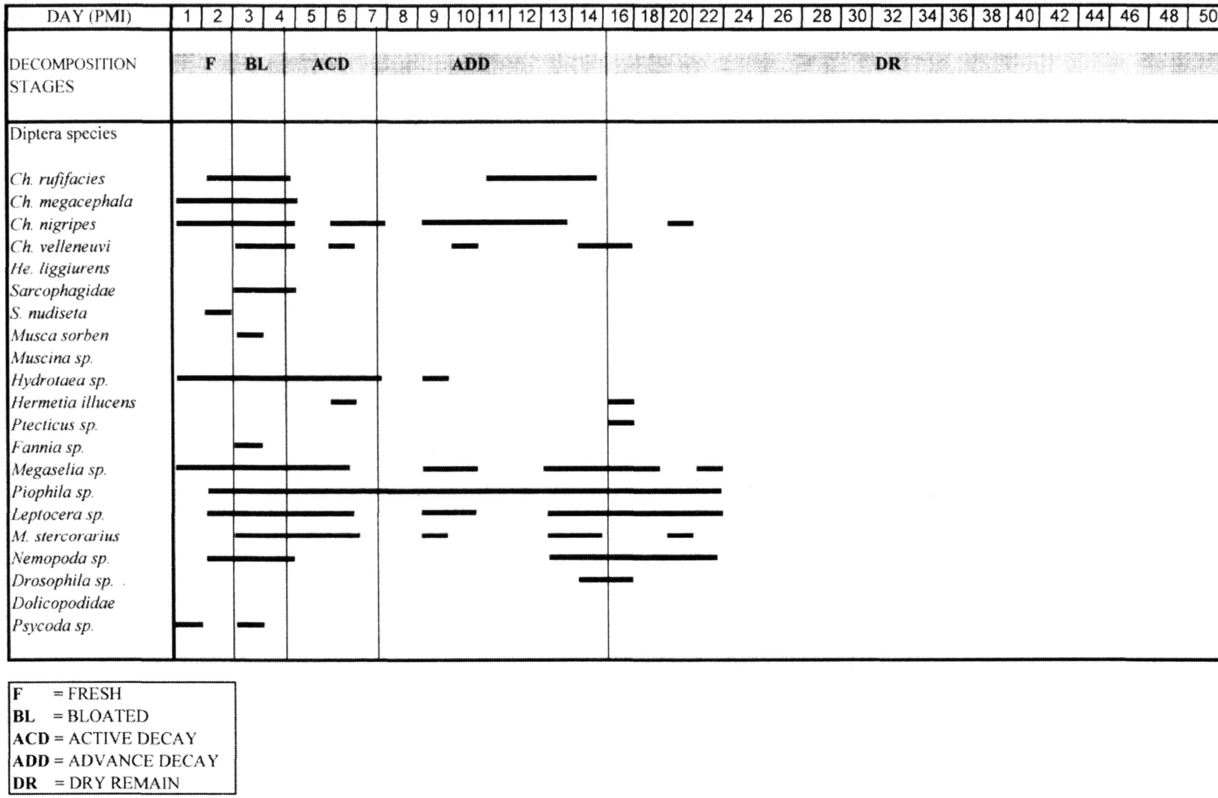


Figure 12: Adult Diptera Succession on Monkey Carcasses in Five Decomposition Stages During Wet Season

In wet season we recorded that carcasses need shorter time to dry. According to Archer (2003), high amount of rainfall can reduce the length of decay stage by increasing mass loss. In addition, rainfall also probably washed away decomposition product and accelerated the time of carcass to dry, thus carcasses tissue was not been interested to the flies anymore. We also suggest that for the tropical region the daily pattern of rainfall in 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 then soaks the tissues. High relative humidity can make tissues becoming a suitable environment for the activity of bacteria. Thus, the amount of rainfall in the first two weeks period of decomposition should be getting more attention rather than monthly cumulative rainfall in studying the decomposition in tropical climate. The rainfall data in two weeks period after dead is important in order to make the PMI estimation.

It has been reported since four decades ago that other than internal anaerobic bacteria, the activity of Diptera larvae of dead body played a significant role in decay process (Payne, 1965; Campobasso, Vella, & Introna, 2001). In this study, even the abundance of larvae were not separated to the taxa, it has showed a relationship in biomass remaining ($p < .05$); thus, it supports the previous statement. It has reported that the larvae on dead body not only consumed tissues as their only food source; for instance the species of *Ch. rufifacies* can prey on *Ch. megacephala* larvae as an alternative food source in high competition conditions (Goodbrod & Goff, 1990).

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

In this study, among the Calliphoridae, the adult of *Ch. nigripes* and *Ch. megacephala* were the earliest species collected from the carcasses in both seasons. However, another blowfly, *Ch. rufifacies*, was recorded that, it arrived later on the second day in both seasons. In Hawaiian Island, several studies indicated that *Ch. rufifacies* was arrived at carrion on the first day of placement of pig carcasses (Early & Goff, 1986; Tullis & Goff, 1987), thus it showed a contradiction to this study. This difference possibly was caused by the predacious ability of *Ch. rufifacies* on carcasses. Only after the presence of *Ch. megacephala* eggs or larvae, adults of *Ch. rufi*facies 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 may be the primary reason why the colonization of adult *Ch. rufifacies* was later than *Ch. megacephala. Leptocera* sp. found on the carcasses at the later stage of decomposition. Martinez, Duque, and Wolff (2006) reported that this species presented at the dry remains stage and was classified as indicator of the dry remains stage.

Throughout the experimental period, adult 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 was also reported that it existed in some Muscidae species, which their competition with Calliphoridae and Sarcophagidae reduced the number of this muscid species (Bharti & Singh, 2003). In previous study, Denno & Cothran (1976) also reported that the competition that existed between adult Calliphorids and Sarcophagids could affect the population size of Sarcophagids.

In wet season, the first appearance of adult *Her. illucens* was recorded on day six of carcasses placement and this resembles to Tomberline, Sheppard, and Joyce (2005). In both seasons, we observed that the presence of this species occurred in the first week period after dead, which is opposites against Lord, Goff, Adkins, and Haskell (1994) finding, which indicated about of 20 to 30 days of postmortem interval for the first colonization of *Her. illucens*. 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 Afandy et al., 2001).

Conclusion and Recommendations

Among the family of Calliphoridae, *Ch. megacephala* Fabricius (Diptera: Calliphoridae) and *Ch. nigripes* Aubertin (Diptera: Calliphoridae) were the earliest necrophagous insects arrived at the carcasses. Wet and dry season influenced the composition and frequencies of Diptera attracted on the carcasses, thus should be highly considered when estimating the postmortem interval using insect succession data. For future research, the study of Diptera composition should be continued with regard to the condition of carcases, such as buried, immersed in water, burning, and hanging since there is no intensive research due to these aspects in Malaysia.

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