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Development patterns of necrophagous flies infesting rabbit carcasses decomposing in Mount Kapur Cave and its surrounding primary forest in Kuching, Sarawak

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Abstract. In addition to the scarcity of forensic entomology baseline data on oviposition of necrophagous insects and completion of their life cycles in the Borneo region, similar data derived from caves remain unreported. Since entomological baseline data can differ from one biogeoclimatic region to another, the lack of such data would limit the practical values of applying entomological evidence in estimating minimum postmortem interval (mPMI). Therefore, this present research that investigated oviposition and completion of life cycles of necrophagous flies infesting rabbit carcasses decomposing in Mount Kapur Cave and its surrounding forest habitat in Kuching, Sarawak merits forensic consideration. In general, 13 taxa of necrophagous flies were identified *viz. Hypopygiopsis violacea*, *Hypopygiopsis fumipennis*, *Hemipyrellia ligurriens*, *Hemipyrellia tagaliana*, *Chrysomya megacephala*, *Chrysomya villeneuvi*, *Chrysomya rufifacies*, *Chrysomya chani*, *Chrysomya pinguis*, *Chrysomya nigripes*, *Ophyra spinigera* and *Ophyra chalcogaster*, as well as unidentified Sarcophagidae. In addition, *Hyp. violacea* and *Hyp. fumipennis* were the two earlier necrophagous flies that oviposited in all rabbit carcasses decomposing in both habitats. While all these necrophagous flies were observed infesting carcasses in Mount Kapur Cave, *Hem. ligurriens* and *Hem. tagaliana* were not found infesting carcasses in the surrounding forest habitat. Complete life cycles for six and five different necrophagous fly species were successfully observed in Mount Kapur Cave and its surrounding forest habitat, respectively. Significant delay in oviposition, as well as longer durations for completing the life cycles in several necrophagous fly species were observed in Mount Kapur Cave when compared with those of surrounding forest habitat ($p < 0.05$). These findings deserve consideration as the first ever forensic empirical baseline data on oviposition and completion of life cycles for necrophagous flies in Sarawak as well as in a cave habitat, in view of its practical values for estimating mPMI for forensic practical caseworks.

INTRODUCTION

Forensic entomology deals with insect evidence for investigating homicide, suicide and suspicious deaths, as well as urban and stored-products for the purpose of law (Rivers and Dahlem, 2014). Insect evidence may also prove useful in investigating cases of human negligence and animal welfare (Gennard, 2012). In addition to its major contribution in the estimation of minimum postmortem interval (mPMI), forensic entomological evidence can also be used to answer forensic questions such as the possible cause of death, relocation of human body and secondary disposal, as well as human identification (Gennard, 2012; Mahat and Jayaprakash, 2013). Pertinently, beyond 72 hours of death, pathological changes (e.g. algor mortis and rigor mortis) might not be able to assist forensic pathologists in determining time of death due to the advanced stages of decomposition (Gennard, 2012). In such situation, forensic entomology may be the only available means for proving or disproving one's alibi (Denis *et al.*, 2018).

While estimation of mPMI can be done by assessing the growth pattern of the oldest necrophagous insects (e.g. the thermal summation approach), interpretation of the insect succession and composition data may be more appropriate for bodies recovered weeks or months after death (Rivers and Dahlem, 2014). In this context, it is pertinent to indicate that the interpretation of entomological evidence relies largely on understanding the oviposition and developmental patterns of necrophagous insects that vary according to variations in biogeoclimatic factors (Goff, 2009) as well as presence of poisons and/or drugs (Mahat *et al.*, 2009; Anderson, 2010). Being poikilotherms, necrophagous insects exhibit holometabolous growth with varying appearances among the different stages of development, dependent on the external source of heat for maintaining its physiological and biochemical processes (Gennard, 2012; Rivers and Dahlem, 2014). Therefore, ambient temperature has been widely reported in the literature as the major factor influencing the duration for completing life cycle, especially in countries with defined seasonal variations such as summer and winter (Sharanowski *et al.*, 2008). Considering that ambient temperature remains largely similar throughout the year in tropical countries like Malaysia, the influence of temperature on insects' development may not be as huge as in temperate countries; other factors such as rainfall (Mahat *et al.*, 2009) may play a bigger role in this forensic context.

In Malaysia, application of entomological evidence is rapidly gaining popularity for forensic practice (Syamsa *et al.*, 2015). Despite the substantial efforts put forth in forensic entomological studies within Peninsular Malaysia, it is evident that such efforts remain geographically imbalanced with limited data originating from East Malaysia (Sarawak and Sabah) (Mahat and Jayaprakash, 2013). Factors necessitating forensic entomological studies in Sarawak and Sabah include the fact that they are (a) geographically separated by the South China Sea, (b) consisted of a larger land area than that of Peninsular Malaysia and (c) perceived as more diverse ecologically in its fauna (Mahat and Jayaprakash, 2013). In view of the indication made by Anderson (2010) that data generated in one biogeoclimatic zone should not be regarded as the same for a different region, specific study focusing on developing empirical baseline data for Sarawak acquires forensic significance. Furthermore, because murder is a crime punishable by capital punishment (death) in many countries including Malaysia (Penal Code (Act 574), 2015), murder concealment acts are generally expected. Murderers may choose secluded locations such as primary forest, abandoned house or even a cave to conceal the victims in their attempts 'to confuse, hamper, or defeat investigative or forensic efforts for the purposes of concealing their identity, their connection to the crime, or the crime itself" (Turvey 2008). Interestingly, review of literature does not reveal any specific study on oviposition and developmental patterns as well as successional patterns of necrophagous flies on corpses/carcasses decomposing in a cave and its surrounding primary forest.

Hence, this present study was aimed at providing for the first time, the empirical baseline data on the developmental patterns of necrophagous flies infesting rabbit carcasses in the two different habitats (*viz.* cave and the surrounding primary forest) in Kuching, Sarawak, in view of its practical values for estimating mPMI as well as paving the way for scientific application of forensic entomology parameters in Borneo region.

MATERIALS AND METHODS

Experimental design

This present research (ACUC approval number: USM/IACUC/2017/(109)(877)) utilized a total of six freshly slaughtered female rabbit carcasses (*Oryctolagus cuniculus*) (~1.5 kg) for studying the varying species of necrophagous insects and durations for completing their life cycles in the Mount Kapur Cave and its surrounding primary forest in Kuching Division of Sarawak. Three carcasses were decomposed in each habitat during 22nd September – 1st November 2017. Purchased as carcasses from a local rabbit meat seller, the rabbits were sacrificed by slaughtering with the front part of the neck severed partially. To prevent exposure towards insects prior to the placement at the decomposition site, the carcasses were transported in separate sealed double plastic bags and placed at respective decomposition sites before 7.00 a.m. Each carcass was placed directly on the ground and separated by a minimum distance of 20 m apart to minimize interruption of flies from the adjacent colonies (Mahat *et al.*, 2014). For preventing disturbance by scavengers especially small mammals yet permitting free access for necrophagous insects, a slotted welded wire mesh cage (45 cm (length) x 42 cm (width) x 60 cm (height)) was used for each carcass. Following the methodology described by Mahat *et al.* (2009; 2014), entomological observations (i.e. the decomposition stages, presence of adult flies, eggs, larvae, pupae, empty pupal cases, tenerals, ambient and carcass surface temperatures, rainfall as well as relative humidity) were recorded daily until the completion of life cycles for all necrophagous flies (until day-41 of decomposition). Exclusively for the cave habitat, daily light intensity was measured.

Decomposition site

The carcasses were decomposed at two different habitats *viz.* in Mount Kapur Cave at Bau, Kuching (1° 22' 50.88" N, 110° 7' 10.46" E, about 70 meters above sea level; altitude.nu, accessed on 24th November 2017) and its surrounding primary forest (1° 22' 56.44" N, 110° 6' 57.42" E, about 40 meters above sea level; altitude.nu, accessed on 24th November 2017) within the Division of Kuching, Sarawak. Mount Kapur Cave is a limestone cave with clay floor, located at a vector displacement of about 5.7 km southwest of the town of Bau, and about 250 meters east of the main entrance of Fairy Cave in Krokong area (www.google.com.my/maps, accessed on 24th November 2017). The study site was an abandoned gold mine, leaving a side chamber occupied by bats (National Resources and Environmental Board, personal communication). On the other hand, the chosen habitat of primary forest of Mount Kapur Cave as defined by the Sarawak Forestry Corporation (personal communication) was located at about 100 m from the nearest human establishment. The vegetation of this primary forest comprised of *Ficus* spp., *Melastoma* spp. and *Calamus* spp. with the estimated canopy coverage of about 80% along with the sparse undergrowth low shrubs, wild ferns and bamboo (Mohd-Azlan, 2005). Three rabbit carcasses were left to decompose in each habitat, separated by the minimum distance of 20 m apart from one another.

Entomological observation and sampling

Field observation was made at every 2-hour interval from 7.00 a.m. to 5.00 p.m. until the first observation of the second instar larvae of the first colonizing necrophagous fly species. Subsequently the observation was continued at every 4-hour interval until the first observation of pupae, twice daily (around 9.00 a.m. and 5 p.m.) during the earlier part of the dry remain stage of decomposition (until day-16) and restricted to once daily (around

noon) until the completion of the 41 days of observation period. In this study, the description of the decomposition process provided by Kreitlow (2010) (*viz*. fresh, bloated, decay, postdecay and remains stages) was used. The entomological observations recorded during every visit included decomposition stages, presence of adult flies, eggs, larvae, pupae, empty pupal cases, tenerals, ambient and carcass surface temperatures, daily total rainfall and its occurrence, as well as percentage relative humidity. Using a digital thermometer, the *in-situ* data logger, a hygrometer, as well as a rain gauge, temperatures (carcass surface and ambient), percentage relative humidity and the daily total rainfall were recorded, respectively. To measure the light intensity in the Mount Kapur Cave, a lux meter was used. For documenting the findings, a digital camera was utilized.

Considering the possible presence of varying species of necrophagous insects on the carcass, representative specimens of larvae (n=10) demonstrating variations in physical appearances (e.g. hairy and the varying sizes of maggots) from all maggot masses, as well as soil underneath and around the carcass were collected using surgical forceps and/or brushes. Half of the larvae sampled were killed by immersing them in hot water (about 80°C) and preserved in specimen containers containing 80% ethanol (Adam and Hall, 2003) for taxonomic identification and ascertaining the larval instar. Following the procedure suggested by Mahat *et al.* (2009), the remaining half of the larvae were reared in plastic rearing cups containing about 3 cm height of soil secured with cotton gauze and rubber bands until the emergence of tenerals for confirming the species. To minimize water retention in the rearing cup, four tiny holes (diameter: about 2 mm) at the bottom of the rearing cup were made. These rearing cups were placed at the same habitat where the specimens were collected and examined for the emergence of tenerals twice daily (around 9 a.m. and 5 p.m.). This was to ensure that the larvae, prepupae and pupae developed at similar ambient conditions with that of surrounding the carcasses. As suggested by Gennard (2012), the number of spiracular slits in the peritremes of posterior spiracles was used for assigning the larval instar. In deciding if a particular stage of development had been achieved, suggestion made by Clarkson *et al.* (2004) was used in this present research. Once the prepupal larvae were observed, the surrounding soil area (a minimum of one-meter radius) was examined for the presence of pupae. These pupae were collected and reared in a rearing cup *in situ* (similar to the rearing cups for larvae) containing sufficient amount of soil to bury the pupae until the emergence of tenerals. The number of pupae placed in each rearing cup shall not exceed 15 for minimizing overcrowding.

Preservation of larvae and tenerals for taxonomic identification

The larvae were partially resected transversely on their 11th segment and soaked in 10% potassium hydroxide (KOH) overnight for dissolving the larval tissues. The larvae then were placed in glacial acetic acid for 15 minutes to neutralize the previous KOH solution. At this time, the gut and muscle contents were removed thoroughly using a minute pin with a wooden applicator stick as its handle. The larvae were then dehydrated in ascending ethanol solutions ranging from 80%, 90%, 95% and 100% for 30 minutes for each solution. The larvae were transferred into clove oil for overnight (for coloring) and soaked in xylene for 30 minutes to wash away the clove oil prior to mounting it using Canada Balsam. As for the adult flies, they were killed using ethyl acetate and pinned with entomological pins for taxonomic identification.

Taxonomic identification of the third instar larvae was made by observing the morphology of the body region and posterior spiracle (Omar, 2002; Sukontason *et al.*, 2004; Ahmad-Firdaus *et al.*, 2010; Heo *et al.*, 2015), while the identification of larval instars was attempted by observing the number of spiracular slits in the peritreme of the posterior spiracles (Gennard, 2012). Upon emergence of tenerals, species identification was made using taxonomic keys provided by Kurahashi *et al.* (1997) and Nazni *et al.* (2011).

Statistical analysis

Statistical analyses were performed using the UTM licensed IBM SPSS 20.0 software. The data analyzed included the ambient temperature, total daily rainfall and duration taken for the onset of initial oviposition as well as the completion of life cycles among the different species of necrophagous flies infesting rabbit carcasses decomposing in the forest and cave habitats. The Shapiro-Wilk test was used for examining the normality of the data. The data were normally distributed whenever the observed p-values for the normality test were greater than 0.05, and vice versa. While the normally distributed data were further analyzed using independent t-test and ANOVA, the non-parametric Mann-Whitney U test and Kruskal-Wallis H were utilized whenever the data violated the assumption of normality. The level of significance (α) of 0.05 was used for inferring any statistical significance.

RESULTS AND DISCUSSION

Environmental condition

Necrophagous flies are poikilotherms (Gennard, 2012; Rivers and Dahlem; 2014), depend largely on environmental heat and hence, their developmental pattern is a factor of ambient temperature (Anderson, 2010; Rivers and Dahlem, 2014). Relative humidity (Krietlow, 2010) and rainfall (Mahat *et al.*, 2009) have also been reported as extrinsic factors influencing oviposition and developmental patterns of necrophagous flies. Therefore, in this present research, the ambient temperature, total daily rainfall (outside of the cave) and percentage relative humidity (Table 1, Figures 1 and 2), as well as light intensity in the cave (Figure 2) were recorded *in situ*. As much as the carcass surface temperatures remained similar among all the three replicate carcasses decomposing in each of the habitats, representative data alone are provided in Figures 1 and 2 for forest habitat as well as in the Mount Kapur Cave, respectively.

Results in Table 1 revealed that the means of ambient temperature during the 41 days of observation period remained similar between the two habitats (Independent Sample t-test, $p > 0.05$). While the minimum and maximum means of ambient temperature in the forest habitat were recorded as 26.51 ± 1.22°C and 27.40 ± 1.42°C, respectively, the same for the cave habitat were observed at 26.41 ± 0.99°C and 27.15 ± 0.66°C, correspondingly. Moreover, total rainfall (> 10.0 mm) in the forest habitat was recorded on day 3, 11–12, 17, 20, 22, 24, 26, 29, 32, 35 and 40 of the decomposition (Figure 1). Pertinently, rains throughout the day were recorded on days-11,17 and 40 of decomposition alone, during which the pupation periods for several of forensically important necrophagous flies were observed in this present research. As for the percentage relative humidity, significant differences between the two habitats were not observed (Independent Sample t-test, $p > 0.05$), despite slightly higher percentages recorded in the cave habitat. The minimum and maximum means relative humidity for the forest habitat being 75.37 ± 9.78% and 88.67 ± 3.35%, respectively; the same for the cave habitat were 80.71 ± 7.11% and 92.22 ± 3.99%, correspondingly (Table 1). The slightly higher mean percentage of relative humidity observed in the cave habitat can be explained by the fact that the water from rain outside of that limestone cave dripped along the stalactites into the cave, wetting its clay floor. Because of the shaded feature of the cave as well as the type of soil, evaporation of the retained water was observably slower when compared with that of forest habitat.

Considering general statements that some insects are positively phototropic (Smith, 1986) 'while others seek carrion in partial or full shade as oviposition sites' (Rivers and Dahlem, 2014), light intensity (lux) was evaluated for the cave habitat. It was found that the light intensity in the cave ranged between 0.75 – 27.40 lux (median: 13.40 lux), depending on the angle of light entering the cave. Although the canopy of trees in the forest habitat may provide limited amount of shade to the carcasses from direct sunlight, the habitat was generally bright throughout the day, and small variations in sunlight intensity may not impede the behaviour of necrophagous insects. Because

Table 1. Means of ambient temperature (°C) and relative humidity (%) at the decomposition site during 22nd September – 1st November 2017

Intervals	Ambient temperature (°C)				Statistical significance between cave and forest	Relative humidity (%)				Statistical significance between cave and forest
	Cave		Forest			Cave		Forest		
	Mean	Range	Mean	Range		Mean	Range	Mean	Range	
22nd Sept – 26th Sept	26.70±0.92	25.09-27.35	27.04±0.96	26.17-28.41	P > 0.05	80.71±7.11	70.60-86.83	84.03±5.03	76.53-90.33	P > 0.05
27th Sept – 1st Oct	26.44±0.36	25.90-26.83	26.51±1.22	24.62-27.93	P > 0.05	86.77±4.07	83.00-91.67	86.09±1.55	84.17-87.67	P > 0.05
2nd Oct – 6th Oct	26.17±0.53	25.33-26.65	26.61±1.36	24.48-28.13	P > 0.05	87.60±1.68	85.67-90.17	86.47±1.43	85.00-88.50	P > 0.05
7th Oct – 11th Oct	27.15±0.66	26.00-27.57	26.89±1.06	25.40-28.17	P > 0.05	84.77±3.44	79.67-88.50	75.37±9.78	65.67-87.83	P > 0.05
12th Oct – 16th Oct	26.99±0.32	26.47-27.27	27.40±1.42	25.77-29.67	P > 0.05	83.33±3.94	80.33-89.67	84.80±5.90	76.00-91.67	P > 0.05
17th Oct – 21st Oct	26.57±1.31	24.50-27.73	26.96±1.09	25.10-27.67	P > 0.05	87.13±2.98	83.67-91.33	88.67±3.35	85.00-94.00	P > 0.05
22nd Oct – 26th Oct	26.41±0.99	25.17-27.67	26.95±0.69	26.03-27.83	P > 0.05	90.47±1.89	85.27-4.31	79.91±4.31	81.00-91.00	P > 0.05
27th Oct – 1st Nov	26.08±0.74	25.00-26.90	27.31±1.22	25.73-29.17	P > 0.05	92.22±3.99	87.6796.67	88.56±6.54	77.00-95.33	P > 0.05

The data on ambient temperature and relative humidity were obtained *in situ* using a digital data logger and a hygrometer, respectively. Statistical analysis using the parametric Independent Sample t-test does not indicate any significant differences (P > 0.05) in both the ambient temperature and relative humidity between the cave and forest habitats throughout the 41 days of observation period.

of extremely limited light intensity in the cave habitat, investigating the effect of low light intensity in the cave habitat on the infesting necrophagous flies was found pertinent than that of in the forest habitat. Apart from light intensity, the amount of shade and the mechanical effect of rain, it can be construed that the carcasses in both habitats were relatively exposed to similar ambient temperature and percentage humidity, as depicted in Figures 1 and 2.

Decomposition stages

One of the biggest issues in forensic entomological studies is choosing the appropriate animal models for decomposition. Although it has been advocated that pig is the best model to represent human decomposition (Wang *et al.*, 2017), other animals like rabbits (Mahat *et al.*, 2009; 2014; 2016) and monkeys (Ahmad and Ahmad, 2009) have also been used. Rabbit carcasses were used in this present research because of their easy handling during entomological observation, but large enough to sustain decomposition to support a considerable number and diversity of carrion insects (Bharti and Singh, 2003). Its choice was also supported by previous researchers (Azwandi *et al.*, 2013) that reported insignificant differences in Shannon-Weiner index (H'), Simpson dominance index (C) and Pielou's

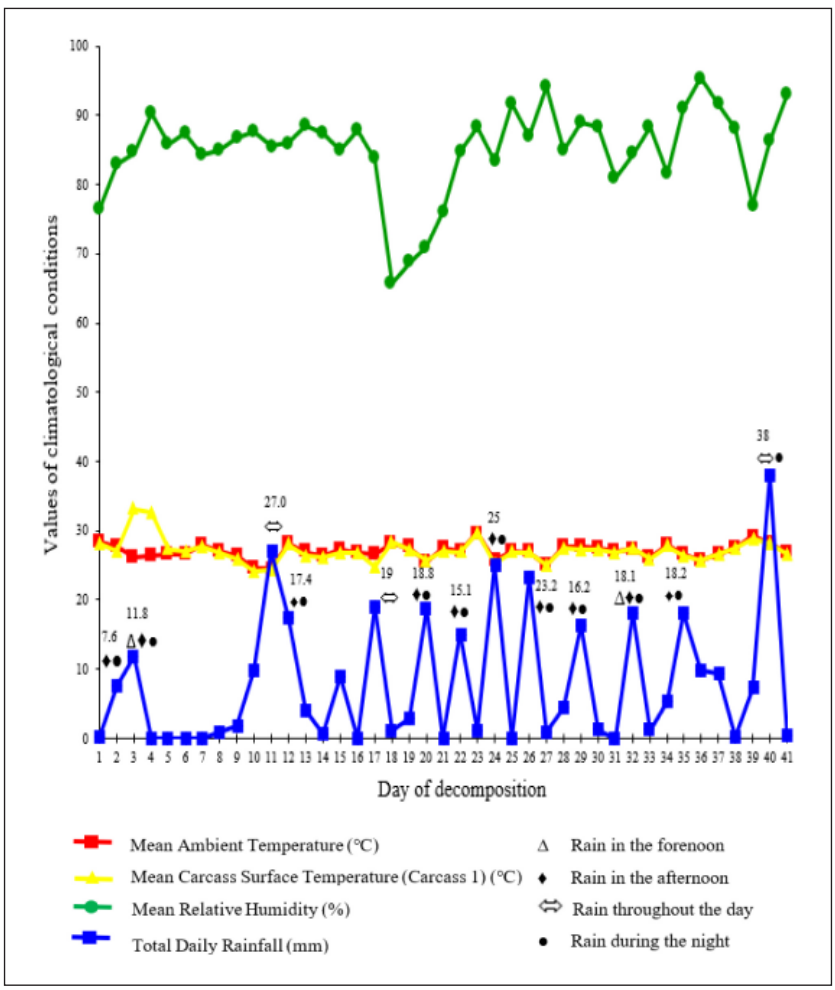

Figure 1. Total daily rainfall (mm) and mean relative humidity (%), as well as means of ambient and representative carcass surface temperatures (°C) during 22nd September – 1st November 2017 in the forest habitat around Mount Kapur Cave, Kuching, Sarawak (1° 22' 56.44" N, 110° 6' 57.42" E).

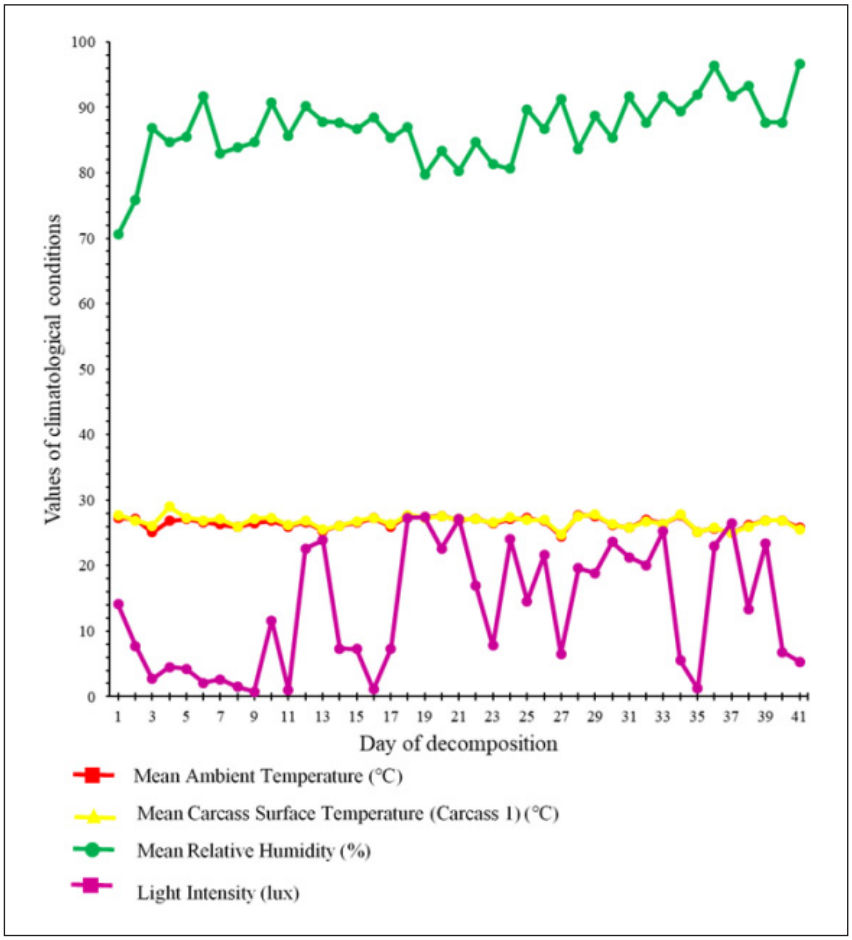

Figure 2. Mean relative humidity (%), means of ambient and representative carcass surface temperatures (°C) as well as light intensity (lux) during 22nd September – 1st November 2017 in Mount Kapur Cave, Kuching, Sarawak (1° 22' 50.88" N, 110° 7' 10.46" E).

Evenness index (J) for the species compositions in rabbit and monkey carcasses. Taking into account all these facts, the choice of utilizing rabbit carcasses made in this present research appears scientifically justifiable.

It has been acknowledged by many forensic entomologists that decomposition process is a continuum of events; however, for providing better understanding on periods of insect activities the process has been divided into several categorical stages (Rivers and Dahlem, 2014). In this context, different insect taxa have been attributed to the different stages of decomposition i.e. the fundamental aspect for succession-based approach for estimating PMI. Interestingly, variations in the number of decomposition stages, as well as their durations have been indicated by many previous forensic entomological studies. While, five stages of decomposition for rabbit carcasses were reported by in Shenzen, China (Wang *et al.*, 2017) and El-Qalyubiya, Egypt (Abd El-bar and Sawaby, 2011), only four stages were reported in Punjab, India (Bharti and Singh, 2003). In this regard, Gennard (2012) attributed variations observed in the number of decomposition stages and their durations to extrinsic factors such as habitats. Among others, the five categorical stages (*viz.* fresh, bloated, decay, postdecay and skeletal or remains) described by Kreitlow (2010) for carcasses decomposing on land have been

acquiring popularity in tropical countries like Malaysia. Table 2 represents the onset and durations (number of days) of the decomposition stages observed in this present research.

In this present research, the five stages of decomposition, recommended by Kreitlow (2010) were also observed in all female rabbit carcasses decomposing in both the forest and cave habitats, the details of which are provided below. The fresh stage of decomposition began from the moment of death until evidence of bloating was observed, and this stage lasted for 24 hours (Table 2) in both habitats. Subsequently, intensification of the catabolism of organic substrates as well as putrefaction by anaerobic bacteria had led to the inflation of the abdomen into balloon-like appearance, known as the bloated stage. Due to the putrefaction and influence of insect activities, the carcass surface temperatures were observed as slightly higher than that of ambient during the bloated stage of decomposition (Figures 1 and 2). During this stage, clusters of dipteran eggs were also found. The bloated stage was observed in all the carcasses in both habitats on day-2 of decomposition, and lasted for about 24 hours (Table 2). The presence of larval feeding masses and bacterial putrefaction had caused deflation of the body of the carcasses, and this signed the onset of decay stage. For rabbit carcasses decomposing in the forest habitat, the decay stage started on day-3 of decomposition, lasted for 48-52 hours and ended by about midday on day-5 of decomposition. Although started on the same day-3 of decomposition, rabbit carcasses decomposing in the cave habitat had significantly longer decay stage (Table 2, Mann-Whitney U test, $p < 0.05$) when compared with that of forest.

Table 2. Day of observation and durations (number of hours) of the individual decomposition stage

Stage	Forest	Cave
Fresh	Day 1 (24)	Day 1 (24)
Bloated	Day 2 (24)	Day 2 (24)
Decay	Day 3-5 (48-52)	Day 3-6 (72-76)*
Postdecay	Day 5-8 (94-96)	Day 6-10 (120-126)*
Remains	Day 9 onwards	Day 11 onwards

* Mann-Whitney U test revealed significantly longer ($p < 0.05$) periods of decay and postdecay stages of decomposition in cave habitat than that of the surrounding forest.

While post decay stage of decomposition for carcasses in forest habitat was observed by about midday on day-5 and lasted until the last observation on day-8, the same for cave habitat took place 24 hours later (midday of day-6) and lasted until end of day-10; the comparison being statistically significant (Table 2, Mann-Whitney U test, $p < 0.05$). The post-decay stage was characterized by the presence of only bone, cartilage and skin, during which dipterans ceased from being the predominant insects on the carcasses. The carcasses were considered entering the remains stage of decomposition when only bones and furs were observed. Considering that this present study was limited until the completion of the life cycle of all necrophagous flies infesting the rabbit carcasses (day-41), the end of the remains stage of decomposition was not observed. The remains stage started on day-9 onwards for carcasses in the forest habitat. On the other hand, the stage started on day-11 onwards for carcasses in the cave habitat. It is pertinent to indicate here that this was the first study reporting about the decomposition stages and their durations for rabbit carcasses decomposing in the limestone cave, limiting suitable comparisons to be made with the relevant literature.

Life cycles of the infesting necrophagous flies

Since for each habitat all the three rabbit carcasses were decomposed in sites that were nearby, it was observed that the life cycles for all the infesting necrophagous flies remained similar, pertained to that habitat. Therefore, the pooled representative data on the life cycles for necrophagous flies in both the cave and forest habitats alone are tabulated here (Tables 3-8). It is pertinent to indicate here that, despite considerable numbers of forensic entomologically-driven studies covering various biogeoclimatic regions in Peninsular Malaysia, specific studies focusing on the Borneo region remain sparse (Mahat and Jayaprakash, 2013).

Table 3. Day and time for the first observation of the different stages of life cycle for *Hypopygiopsis violacea* in Mount Kapur cave and forest habitats

Replicates	Habitat	First observation							
		Eggs	**1st instar larvae**	**2nd instar larvae**	**3rd instar larvae**	**Post feeding larvae**	**Pupae**	**Tenerals**	**Total duration from eggs to tenerals**
1	Cave	Day-2 (7 a.m.) [24 h]	Day-2 (5 p.m.) [10 h]	Day-3 (9 a.m.) [16 h]	Day-4 (9 a.m.) [24 h]	Day-5 (11 a.m.) [26 h]	Day-25 (5 p.m.) [486 h]	Day-40 (11 a.m.) [354 h]	918 hours/ 38.25 days
	Forest	Day-1 (9 a.m.) [2 h]	Day-2 (7 a.m.) [22 h]	Day-2 (5 p.m.) [10 h]	Day-3 (3 p.m.) [22 h]	Day-4 (9 a.m.) [18 h]	Day-10 (5 p.m.) [152 h]	Day-24 (11 a.m.) [330 h]	554 hours/ 23.08 days
2	Cave	Day-2 (7 a.m.) [24 h]	Day-2 (5 p.m.) [10 h]	Day-3 (9 a.m.) [16 h]	Day-4 (9 a.m.) [24 h]	Day-5 (11 a.m.) [26 h]	Day-25 (5 p.m.) [486 h]	Day-40 (11 a.m.) [354 h]	918 hours/ 38.25 days
	Forest	Day-1 (9 a.m.) [2 h]	Day-2 (7 a.m.) [22 h]	Day-2 (5 p.m.) [10 h]	Day-3 (3 p.m.) [22 h]	Day-4 (11 a.m.) [20 h]	Day-11 (9 a.m.) [166 h]	Day-25 (11 a.m.) [338 h]	578 hours/ 24.08 days
3	Cave	Day-2 (7 a.m.) [24 h]	Day-2 (5 p.m.) [10 h]	Day-3 (11 a.m.) [18 h]	Day-4 (7 a.m.) [20 h]	Day-5 (3 p.m.) [32 h]	Day-26 (9 a.m.) [498 h]	Day-40 (11 a.m.) [338 h]	918 hours/ 38.25 days
	Forest	Day-1 (9 a.m.) [2 h]	Day-2 (7 a.m.) [22]	Day-2 (5 p.m.) [10 h]	Day 3 (3 p.m.) [22 h]	Day-4 (9 a.m.) [18 h]	Day-10 (5 p.m.) [152 h]	Day-24 (11 a.m.) [330 h]	554 hours/ 23.08 days
Range	Cave	(7 a.m.-7 a.m.)	(5 p.m.-5 p.m.)	(9 a.m.-11 a.m.)	(7 a.m.-9 a.m.)	(11 a.m.-3 p.m.)	(5 p.m.-9 a.m.)	(11 a.m.-11 a.m.)	918-918 hours/ 38.25-38.25 days
	Forest	(9 a.m.-9 a.m.)	(7 a.m.-7 a.m.)	(5 p.m.-5 p.m.)	(3 p.m.-3 p.m.)	(9 a.m.-11 a.m.)	(5 p.m.-11 a.m.)	(11 a.m.-11 a.m.)	554-578 hours/ 23.08-24.08 days

Values in brackets ( ) and square parentheses [ ] indicate the time and duration in hours for the first observation of a particular stage of development, respectively.

Table 4. Day and time for the first observation of the different stages of life cycle for *Hypopygiopsis fumipennis* in Mount Kapur cave and forest habitats

Replicates	Habitat	First Observation							
		Eggs	**1st instar larvae**	**2nd instar larvae**	**3rd instar larvae**	**Post feeding larvae**	**Pupae**	**Tenerals**	**Total duration from eggs to tenerals**
1	Cave	Day-2 (7 a.m.) [24 h]	Day-2 (5 p.m.) [10 h]	Day-3 (9 a.m.) [16 h]	Day-4 (9 a.m.) [24 h]	Day-5 (5 p.m.) [32 h]	Day-31 (11 a.m.) [618 h]	Day-41 (9 a.m.) [238 h]	938 hours/ 39.08 days
	Forest	Day-1 (9 a.m.) [2 h]	Day-1 (5 p.m.) [8 h]	Day-2 (7 a.m.) [14 h]	Day-3 (3 p.m.) [32 h]	Day-5 (9 a.m.) [42 h]	Day-25 (5 p.m.) [488 h]	Day-40 (11 a.m.) [354 h]	938 hours/ 39.08 days
2	Cave	Day-2 (7 a.m.) [24 h]	Day-2 (5 p.m.) [10 h]	Day-3 (9 a.m.) [16 h]	Day-4 (9 a.m.) [24 h]	Day-5 (5 p.m.) [32 h]	Day-31 (11 a.m.) [618 h]	Day-41 (9 a.m.) [238 h]	938 hours/ 39.08 days
	Forest	Day-1 (9 a.m.) [2 h]	Day-1 (5 p.m.) [8 h]	Day-2 (7 a.m.) [14 h]	Day-3 (3 p.m.) [32 h]	Day-5 (9 a.m.) [42 h]	Day-25 (5 p.m.) [488 h]	Day-40 (11 a.m.) [354 h]	938 hours/ 39.08 days
3	Cave	Day-2 (7 a.m.) [24 h]	Day-2 (5 p.m.) [10 h]	Day-3 (9 a.m.) [16 h]	Day-4 (9 a.m.) [24 h]	Day-5 (5 p.m.) [32 h]	Day-31 (11 a.m.) [618 h]	Day-41 (9 a.m.) [238h]	938 hours/ 39.08 days
	Forest	Day-1 (9 a.m.) [2 h]	Day-1 (5 p.m.) [8 h]	Day-2 (7 a.m.) [14 h]	Day-3 (3 p.m.) [32 h]	Day-5 (9 a.m.) [42 h]	Day-25 (5 p.m.) [488 h]	Day-40 (11 a.m.) [354 h]	938 hours/ 39.08 days
Range	Cave	(7 a.m.-7 a.m.)	(5 p.m.-5 p.m.)	(9 a.m.-9 a.m.)	(9 a.m.-9 a.m.)	(5 p.m.-5 p.m.)	(11 a.m.-11 a.m.)	(9 a.m.-9 a.m.)	938-938 hours/ 39.08-39.08 days
	Forest	(9 a.m.-9 a.m.)	(5 p.m.-5 p.m.)	(7 a.m.-7 a.m.)	(3 p.m.-3 p.m.)	(9 a.m.-9 a.m.)	(5 p.m.-5 p.m.)	(11 a.m.-11 a.m.)	938-938 hours/ 39.08-39.08 days

Values in brackets ( ) and square parentheses [ ] indicate the time and duration in hours for the first observation of a particular stage of development, respectively.

Table 5. Day and time for the first observation of the different stages of life cycle for *Chrysomya megacephala* in Mount Kapur cave and forest habitats

Replicates	Habitat	First Observation							
		Eggs	**1st instar larvae**	**2nd instar larvae**	**3rd instar larvae**	**Post feeding larvae**	**Pupae**	**Tenerals**	**Total duration from eggs to tenerals**
1	Cave	Day-2 (1 p.m.) [30 h]	Day-3 (7 a.m.) [30 h]	Day-3 (5 p.m.) [10 h]	Day-4 (9 a.m.) [16 h]	Day-5 (5 p.m.) [32 h]	Day-6 (11 a.m.) [18 h]	Day-12 (9 a.m.) [142 h]	248 hours/ 10.33 days
	Forest	Day-1 (11 a.m.) [4 h]	Day-1 (5 p.m.) [6 h]	Day-2 (7 a.m.) [14 h]	Day-3 (3 p.m.) [32 h]	Day-4 (9 a.m.) [18 h]	Day-5 (5 p.m.) [32 h]	Day-11 (11 a.m.) [138 h]	240 hours/ 10.00 days
2	Cave	Day-2 (11 a.m.) [30 h]	Day-3 (7 a.m.) [32 h]	Day-3 (3 p.m.) [8 h]	Day-4 (7 a.m.) [16 h]	Day-5 (5 p.m.) [32 h]	Day-6 (3 p.m.) [22 h]	Day-12 (9 a.m.) [138 h]	248 hours/ 10.33 days
	Forest	Day-1 (11 a.m.) [4 h]	Day-1 (5 p.m.) [6 h]	Day-2 (9 a.m.) [16 h]	Day-3 (5 p.m.) [32 h]	Day-4 (7 a.m.) [16 h]	Day-5 (5 p.m.) [34 h]	Day-11 (11 a.m.) [138 h]	242 hours/ 10.08 days
3	Cave	Day-2 (1 p.m.) [30 h]	Day-3 (7 a.m.) [30 h]	Day-3 (5 p.m.) [10 h]	Day-4 (9 a.m.) [16 h]	Day-5 (5 p.m.) [32 h]	Day-6 (11 a.m.) [30 h]	Day-12 (9 a.m.) [142 h]	260 hours/ 10.83 days
	Forest	Day-1 (11 a.m.) [4 h]	Day-1 (5 p.m.) [6 h]	Day-2 (9 a.m.) [16 h]	Day-3 (3 p.m.) [30 h]	Day-4 (7 a.m.) [18 h]	Day-5 (5 p.m.) [34 h]	Day-11 (11 a.m.) [138 h]	242 hours/ 10.08 days
Range	Cave	(11 a.m.-1 p.m.)	(7 a.m.-7 a.m.)	(3 p.m.-5 p.m.)	(7 a.m.-9 a.m.)	(5 p.m.-5 p.m.)	(11 a.m.-3 p.m.)	(9 a.m.-9 a.m.)	248-260 hours/ 10.33-10.83 days
	Forest	(11 a.m.-11 a.m.)	(5 p.m.-5 p.m.)	(7 a.m.-9 a.m.)	(3 p.m.-5 p.m.)	(7 a.m.-9 a.m.)	(5 p.m.-5 p.m.)	(11 a.m.-11 a.m.)	240-242 hours/ 10.00-10.08 days

Values in brackets ( ) and square parentheses [ ] indicate the time and duration in hours for the first observation of a particular stage of development, respectively.

Table 6. Day and time for the first observation of the different stages of life cycle for *Chrysomya villeneuvi* in Mount Kapur cave and forest habitats

Replicates	Habitat	First Observation						
		1st instar larvae	**2nd instar larvae**	**3rd instar larvae**	**Post feeding larvae**	**Pupae**	**Tenerals**	**Total duration from eggs to tenerals**
1	Cave	Day-3 (7 a.m.) [14 h]	Day-4 (11 a.m.) [28 h]	Day-5 (11 a.m.) [26 h]	Day-8 (9 a.m.) [70 h]	Day-9 (5 p.m.) [32 h]	Day-15 (9 a.m.) [136 h]	306 hours/ 12.75 days
	Forest	Day-2 (7 a.m.) [14]	Day-2 (5 p.m.) [10 h]	Day 3 (9 a.m.) [16 h]	Day-8 (9 a.m.) [120]	Day-9 (9 a.m.) [24 h]	Day-13 (5 p.m.) [104]	288 hours/ 12.00 days
2	Cave	Day-3 (9 a.m.) [16 h]	Day-4 (11 a.m.) [26 h]	Day-5 (3 p.m.) [28 h]	Day-8 (5 p.m.) [74 h]	Day-10 (9 a.m.) [40 h]	Day-15 (5 p.m.) [128 h]	312 hours/ 13.00 days
	Forest	Day-2 (7 a.m.) [14 h]	Day-2 (5 p.m.) [10 h]	Day-3 (7 a.m.) [14 h]	Day-8 (9 a.m.) [122 h]	Day-9 (9 a.m.) [24 h]	Day-13 (9 a.m.) [96 h]	280 hours/ 11.67 days
3	Cave	Day-3 (9 a.m.) [16]	Day-4 (11 a.m.) [26 h]	Day-5 (3 p.m.) [28 h]	Day-8 (5 p.m.) [74 h]	Day-10 (9 a.m.) [40 h]	Day-15 (5 p.m.) [128 h]	312 hours/ 13.00 days
	Forest	Day-2 (9 a.m.) [16 h]	Day-2 (5 p.m.) [8 h]	Day-3 (9 a.m.) [16 h]	Day-8 (9 a.m.) [120 h]	Day-9 (9 a.m.) [24 h]	Day-14 (9 a.m.) [120 h]	304 hours/ 12.67 days
Range	Cave	(7 a.m.-9 a.m.)	(11 a.m.-11 a.m.)	(11 a.m.-3 p.m.)	(9 a.m.-5 p.m.)	(5 p.m.-9 a.m.)	(9 a.m.-5 p.m.)	306-312 hours/ 12.75-13.00 days
	Forest	(7 a.m.-9 a.m.)	(5 p.m.-5 p.m.)	(7 a.m.-9 a.m.)	(9 a.m.-9 a.m.)	(9 a.m.-9 a.m.)	(9 a.m.-5 p.m.)	280-304 hours/ 11.67-12.67 days

Values in brackets ( ) and square parentheses [ ] indicate the time and duration in hours for the first observation of a particular stage of development, respectively.

Table 7. Day and time for the first observation of the different stages of life cycle for *Chrysomya rufifacies* in Mount Kapur cave and forest habitats

Replicates	Habitat	First Observation						
		1st instar larvae	**2nd instar larvae**	**3rd instar larvae**	**Post feeding larvae**	**Pupae**	**Tenerals**	**Total duration from eggs to tenerals**
1	Cave	Day-3 (9 a.m.) [16 h]	Day-3 (5 p.m.) [8 h]	Day-4 (7 a.m.) [14 h]	Day-5 (3 p.m.) [32 h]	Day-9 (5 p.m.) [96 h]	Day-13 (5 p.m.) [96 h]	296 hours/ 12.33 days
	Forest	Day-2 (9 a.m.) [16 h]	Day-2 (5 p.m.) [8 h]	Day-3 (9 a.m.) [16 h]	Day-4 (3 p.m.) [30 h]	Day-8 (5 p.m.) [98 h]	Day-12 (9 a.m.) [88 h]	266 hours/ 11.08 days
2	Cave	Day-3 (9 a.m.) [16 h]	Day-3 (5 p.m.) [8 h]	Day-4 (9 a.m.) [16 h]	Day-5 (3 p.m.) [30 h]	Day-9 (5 p.m.) [98 h]	Day-13 (5 p.m.) [96 h]	298 hours/ 12.42 days
	Forest	Day-2 (9 a.m.) [16 h]	Day-2 (5 p.m.) [8 h]	Day-3 (7 a.m.) [14 h]	Day-4 (1 p.m.) [28 h]	Day-8 (5 p.m.) [100 h]	Day-12 (9 a.m.) [88 h]	264 hours/ 11.00 days
3	Cave	Day-3 (9 a.m.) [16 h]	Day-3 (5 p.m.) [8 h]	Day-4 (9 a.m.) [16 h]	Day-5 (5 p.m.) [32 h]	Day-9 (5 p.m.) [96 h]	Day-13 (5 p.m.) [96 h]	298 hours/ 12.42 days
	Forest	Day-2 (9 a.m.) [16 h]	Day-2 (5 p.m.) [8 h]	Day-3 (9 a.m.) [16 h]	Day-4 (1 p.m.) [28 h]	Day-8 (5 p.m.) [100 h]	Day-12 (9 a.m.) [88 h]	266 hours/ 11.08 days
Range	Cave	(9 a.m.-9 a.m.)	(5 p.m.-5 p.m.)	(7 a.m.-9 a.m.)	(3 p.m.-5 p.m.)	(5 p.m.-5 p.m.)	(5 p.m.-5 p.m.)	296-298 hours/ 12.33-12.42 days
	Forest	(9 a.m.-9 a.m.)	(5 p.m.-5 p.m.)	(7 a.m.-9 a.m.)	(1 p.m.-3 p.m.)	(5 p.m.-5 p.m.)	(9 a.m.-9 a.m.)	264-266 hours/ 11.00-11.08 days

Values in brackets ( ) and square parentheses [ ] indicate the time and duration in hours for the first observation of a particular stage of development, respectively.

Table 8. Day and time for the first observation of the different stages of life cycle for *Hemipyrellia ligurriens* in Mount Kapur cave and forest habitats

Replicates	Habitat	First Observation						
		1st instar larvae	**2nd instar larvae**	**3rd instar larvae**	**Post feeding larvae**	**Pupae**	**Tenerals**	**Total duration from eggs to tenerals**
1	Cave	Day-2 (5 p.m.) [10 h]	Day-3 (7 a.m.) [14 h]	Day-3 (5 p.m.) [10 h]	Day-5 (1 p.m.) [44 h]	Day-7 (9 a.m.) [44 h]	Day-23 (11 a.m.) [386 h]	532 hours/ 22.17 days
	Forest	Not observed						
2	Cave	Day-2 (5 p.m.) [10 h]	Day-3 (7 a.m.) [14 h]	Day-3 (5 p.m.) [10 h]	Day-5 (1 p.m.) [44 h]	Day-7 (9 a.m.) [44 h]	Day-23 (11 a.m.) [386 h]	532 hours/ 22.17 days
	Forest	Not observed						
3	Cave	Day-2 (5 p.m.) [10 h]	Day-3 (7 a.m.) [14 h]	Day-3 (5 p.m.) [10 h]	Day-5 (1 p.m.) [44 h]	Day-7 (9 a.m.) [44 h]	Day-23 (11 a.m.) [386 h]	532 hours/ 22.17 days
	Forest	Not observed						
Range	Cave	(5 p.m.-5 p.m.)	(7 a.m.-7 a.m.)	(5 p.m.-5 p.m.)	(1 p.m.-1 p.m.)	(9 a.m.-9 a.m.)	(11 a.m.-11 a.m.)	532-532 hours/ 22.17-22.17 days
	Forest	Not observed						

Values in brackets ( ) and square parentheses [ ] indicate the time and duration in hours for the first observation of a particular stage of development, respectively.

Review of literature reveals only one specific study in Borneo reporting the species of adult flies of forensic importance from two peat swamp areas in Kuching, Sarawak (Maramat and Abdul-Rahim, 2015). Considering that species composition and durations for completing life cycle for necrophagous insects can vary due to differences in biogeoclimatic regions, estimation of PMI based on baseline data derived from one region for use in another can be erroneous (Anderson, 2010). Because similar studies focusing on cave habitats, as well as primary forest in Sarawak are unavailable, this present research that was conducted in such habitats as Mount Kapur Cave and its surrounding primary forest in Kuching, Sarawak, appears forensically relevant.

It is pertinent to note that 13 taxa of necrophagous flies were identified *viz*. *Hypopygiopsis violacea*, *Hypopygiopsis fumipennis*, *Hemipyrellia ligurriens*, *Hemipyrellia tagaliana*, *Chrysomya megacephala*, *Chrysomya villeneuvi*, *Chrysomya rufifacies*, *Chrysomya chani*, *Chrysomya pinguis*, *Chrysomya nigripes*, *Ophyra spinigera* and *Ophyra chalcogaster*, as well as an unidentified Sarcophagidae were found infesting the carcasses. However, complete lifecycles were only successfully observed in six (*Hypopygiopsis violacea* (Macquart), *Hypopygiopsis fumipennis* (Walker), *Hemipyrellia ligurriens* (Wiedermann), *Chrysomya megacephala* (Fabricius), *Chrysomya villeneuvi* (Patton) and *Chrysomya rufifacies* (Macquart)) and five (with exception of *Hem. ligurriens*) species in Mount Kapur Cave and its surrounding forest, respectively (Tables 3-8). As for other species, they were either found as isolated larvae, pupae or tenerals, and therefore, specifying their time of arrival on the decomposing rabbits was not possible. The inability to categorically record the completion of lifecycles for them can be attributable to the geographical conditions of the cave (dark) and forest (hilly and covered with vegetation) that rendered difficulties in entomological observation.

Suitable statistical comparisons between the life cycles of necrophagous flies observed in Mount Kapur Cave when compared with that of its surrounding forest habitat are provided in Tables 9 and 10. Unfortunately, statistical comparison for specimens of Sarcophagidae between Mount Kapur Cave and its surrounding forest habitats was not able to be attempted due to inability to taxonomically identify their species. This is consistent with indications made by previous researchers that identification of sarcophagid flies as well as their larvae is difficult due to limited taxonomic keys (Gennard, 2012; Rivers and Dahlem, 2014). Furthermore, the complete life cycle for Sarcophagidae in the surrounding forest habitat was unable to be observed.

In this present research, *Hyp. violacea* and *Hyp. fumipennis* were invariably observed as the first two necrophagous species that oviposited on rabbit carcasses decomposing in both the Mount Kapur Cave and the surrounding primary forest habitats in Kuching, Sarawak (Tables 3 and 4). This finding is consistent with that reported by previous researchers (Omar *et al.*, 1994a; Chen *et al.*, 2008) on monkey carcasses decomposing in a rubber plantation and a tropical rainforest in Peninsular Malaysia. In this context, quotations that 'both species which are considered the least studied and are the only two species recorded in this country' (Silahuddin *et al.*, 2015) and '*Hypopygiopsis* as its biology and ecology are largely unknown' (Heo *et al.*, 2015) appear relevant to this present research, justifying for focusing on these two species. Because review of literature does not reveal any field experiments in Malaysia as well as its neighboring countries that explore the durations for completing the stages of life cycles for *Hyp. violaceae* and *Hyp. fumipennis*, suitable comparisons with the findings reported here cannot be made. The only available data pertaining to these two necrophagous species were obtained from laboratory-controlled experiments (Chen *et al.*, 2008; Heo *et al.*, 2015).

While oviposition by both *Hyp. violaceae* and *Hyp. fumipennis* in the surrounding forest habitat was observed by 9 a.m. on the first day of decomposition, the same was statistically delayed (Table 9, $p < 0.05$) to 7 a.m. to the next day (day-2) in Mount

Table 9. Comparisons of the durations (hours) for the onset of the different stages of life cycles for the infesting *Hyp. violacea, Hyp. fumipennis and C. megacephala* on carcasses decomposing in forest and cave habitats

Stages of life cycle	*Hyp. violaceae*		*Hyp. fumipennis*		*C. megacephala*	
	Forest	**Cave**	**Forest**	**Cave**	**Forest**	**Cave**
Placement until oviposition	2.0 (2.0-2.0)	24.0^{SA} (24.0-24.0)	2.0 (2.0-2.0)	24.0^{SA} (24.0-24.0)	4.0 (4.0-4.0)	30.0^{SA} (30.0-30.0)
Eggs to 1st instar	22.0^{SA} (22.0-22.0)	10.0^{SA} (10.0-10.0)	8.0 (8.0-8.0)	10.0^{SA} (10.0-10.0)	6.0 (6.0-6.0)	30.0^{SA} (30.0-32.0)
1st to 2nd instar	10.0 (10.0-10.0)	16.0^{SA} (16.0-18.0)	14.0 (14.0-14.0)	16.0^{SA} (16.0-16.0)	15.33 ± 1.15^{SB} (14.0-16.0)	9.33 ± 1.15 (8.0-10.0)
2nd to 3rd instar	22.0 ± 0.0 (22.0-22.0)	22.67 ± 2.31 (20.0-24.0)	32.0^{S} (32.0-32.0)	24.0 (24.0-24.0)	32.0^{SA} (30.0-32.0)	16.0 (16.0-16.0)
3rd instar to post feeding larvae	18.67 ± 1.15 (18.0-20.0)	28.0 ± 3.46^{SB} (26.0-32.0)	42.0^{S} (42.0-42.0)	32.0 (32.0-32.0)	18.0 (16.0-18.0)	32.0^{SA} (32.0-32.0)
Post feeding larvae to pupae	152.0 (152.0-166.0)	486.0^{SA} (486.0-498.0)	488.0 (488.0-488.0)	618.0^{SA} (618.0-618.0)	33.33 ± 1.15 (32.0-34.0)	23.33 ± 6.11 (18.0-30.0)
Pupae to tenerals	332.67 ± 4.62 (330.0-338.0)	355.33 ± 2.31^{SB} (354.0-358.0)	354.0^{S} (354.0-354.0)	238.0 (238.0-238.0)	138.0 (138.0-138.0)	142.0 (138.0-142.0)
Duration for completion	554.0 (554.0-578.0)	918.0^{SA} (918.0-918.0)	938.0 (938.0-938.0)	938.0 (938.0-938.0)	241.33 ± 1.15 (240.0-242.0)	252.0 ± 6.93 (248.0-260.0)

Parameters analyzed using Mann-Whitney U Test (^{A}) and Independent Sample t Test (^{B}) are presented as median (range) and mean ± standard deviation, respectively. Level of significance of 0.05 is used for assigning statistical significance (^{S}).

Table 10. Comparisons of the durations (hours) for the onset of the different stages of life cycles for the infesting *C. villeneuvi* and *C. rufifacies* on carcasses decomposing in forest and cave habitats

Stages of life cycle	*C. villeneuvi*		*C. rufifacies*	
	Forest	**Cave**	**Forest**	**Cave**
Eggs to 1st instar	14.0 (14.0-16.0)	16.0^{SA} (14.0-16.0)	16.0 (16.0-16.0)	16.0(16.0-16.0)
1st to 2nd instar	9.3 ± 1.15 (8.0-10.0)	26.67 ± 1.15^{SB} (26.0-28.0)	8.0 (8.0-8.0)	8.0 (8.0-8.0)
2nd to 3rd instar	15.3 ±1.15 (14.0-16.0)	27.33 ± 1.15^{SB} (26.0-28.0)	16.0 (14.0-16.0)	16.0 (14.0-16.0)
3rd instar to postfeeding larvae	120^{SA} (120.0-122.0)	74.0 (70.0-74.0)	28.67 ±1.15 (28.0-30.0)	31.33 ± 1.15^{SB} (30.0-32.0)
Post-feeding larvae to pupae	24.0 (24.0-24.0)	40.0^{SA} (32.0-40.0)	99.33 ± 1.15^{S} (98.0-100.0)	96.67 ± 1.15 (96.0-98.0)
Pupae to tenerals	106.67 ± 12.2 (96.0-120.0)	$130.67 \pm 4.6\ 2^{SB}$ (128.0-136.0)	88.0 (88.0-88.0)	96.0^{SA} (96.0-96.0)
Duration for completion	290.67 ± 12.2 (280.0-304.0)	310.0 ± 3.46 (306.0-312.0)	266.0 (264.0-266.0)	298.0^{SA} (296.0-298.0)

Parameters analyzed using Mann-Whitney U Test (^{A}) and Independent Sample t Test (^{B}) are presented as median (range) and mean ± standard deviation, respectively. Level of significance of 0.05 is used for assigning statistical significance (^{S}).

Kapur Cave (Tables 4 and 5). The delayed oviposition by these two necrophagous species in the carcasses in Mount Kapur Cave can be attributed to the fact that necrophagous flies are diurnal, whereby they are more active in habitat with the presence of light sources (Gennard, 2012; Rivers and Dahlem, 2015) than that of dark habitat, as well as in the night (Pritam and Jayaprakash, 2009). In this situation, oviposition on carcasses in the Mount Kapur Cave occurred during the bloated stage of decomposition that was associated with the presence of intense decomposition odor. On the other hand, oviposition by these two species observed in carcasses decomposing in the surrounding forest habitat occurred during the fresh stage of decomposition, probably attributable to their ready availability in the habitat. As for the first, second and third instar larvae, as well as the postfeeding larvae of *Hyp. violaceae*, the stages were observed on day-2 (7 a.m.), day-2 (5 p.m.), day-3 (3 p.m.) and day-4 (9-11 a.m.) in carcasses decomposing in the surrounding forest habitat, respectively. As for the carcasses in the Mount Kapur Cave, the same stages for *Hyp. violaceae* were observably delayed by about one day than those in the forest habitat, attributable to the delayed oviposition observed earlier (Tables 3 and 9). In addition, significantly longer periods of postfeeding larvae-pupae and pupae-tenerals, as well as the total duration for completing the life cycle for *Hyp. violaceae* were observed in the Mount Kapur Cave (918 hours, 38.25 days) when compared with that of the surrounding forest habitat (554-578 hours, 23.08-24.08 days) (Tables 3-4 and 9, $p < 0.05$).

Interestingly, while no significant difference in the total duration for completing the life cycle for *Hyp. fumipennis* between the two habitats was observed (938 hours, 39.08 days), significantly longer durations for the emergence of third instar larvae (from the second instar) as well as tenerals (from the pupae) were observed in the surrounding forest habitat than that of Mount Kapur Cave (Table 9, $p < 0.05$). The longer durations observed during such stages may be attributable to the presence of heavy rains (Figure 1: day-2, 3, 26, 29, 32, 35 and 40) during such period and/ or the night before that rendered the soil at the habitat soaked with water. This argument is consistent with the indications made by previous researchers that 'rain-soaked soil delays development' (Greenberg and Kunich, 2002) and 'rainfall of about 9.0 mm or more a day during the period of pupation that rendered the soil wet consistently prolonged the period of pupation' (Mahat *et al.*, 2009).

The total duration for completing the life cycle for *Hyp. violaceae* observed in this present research was substantially longer from that of 308.25 ± 8.25 hours (equivalent to 12-13 days) reported by Chen *et al.* (2008) in their laboratory-controlled experiment. In contrast, the duration for completing the life cycle for *Hyp. fumipennis* in this present research was shorter than the 62-72 days reported by Heo *et al.* (2015) in their laboratory. Interestingly, Heo *et al.* (2015) also reported a personal communication with the Institute for Medical Research, Kuala Lumpur indicating that the completion of life cycle for this particular necrophagous species in the insectarium being 19-23 days. The variations observed here when compared with the two laboratory-controlled experiments can be explained by fluctuation of the environmental conditions that prevailed at the decomposition sites, as well as other extrinsic factors that merit further research.

In contrast to the findings reported in Peninsular Malaysia (Heo *et al.*, 2007; Mahat and Jayaprakash, 2013; Mahat *et al.*, 2009; 2014; 2016, Denis *et al.*, 2018), *C. megacephala* was not found as the first and dominant necrophagous fly to oviposit in rabbit carcasses decomposing in Mount Kapur Cave and the surrounding forest habitats. Although oviposition by *C. megacephala* was observed on the first day of decomposition in the forest habitat (11 a.m.) (Table 5), the event occurred later than that of *Hyp. violaceae* and *Hyp. fumipennis*. Nonetheless, the observation of oviposition by *C. megacephala* on the first day of decomposition remains consistent with previous findings reported in Peninsular Malaysia (Heo *et al.*, 2007; Mahat and Jayaprakash, 2013; Mahat *et al.*, 2009; 2014;

2016; Denis *et al.*, 2018). The same pattern of delayed oviposition for *Hyp. violaceae* and *Hyp. fumipennis* observed in the Mount Kapur Cave was also evident for *C. megacephala*. Oviposition of *C. megacephala* in the Mount Kapur Cave was observed between 11 a.m. to 1 p.m. on day-2 of decomposition i.e. at median of 30 hours when compared with only 4 hours in the forest habitat (Table 9, $p < 0.05$). The earliest observations of the first, second and third instar larvae of *C. megacephala* in the surrounding forest habitat were recorded on day-1 (5 p.m.), day-2 (7-9 a.m.) and day-3 (3-5 p.m.), respectively (Table 5). In contrast, the same stages of larvae were seen on day-3 (7 a.m.), day-3 (3-5 p.m.) and day-4 (7-9 a.m.), correspondingly in Mount Kapur Cave (Table 4). Significantly longer periods of first-second instar (15.33 ± 1.15 hours) and second-third instar larvae (32 (30-32) hours) of *C. megacephala* were observed in the surrounding forest habitat when compared with that in Mount Kapur Cave (Table 9, $p < 0.05$). The prolongation of these stages may be due to the rain during the night before (7.6 mm), as well as heavy rain during the day of observation (11.8 mm, afternoon until the next morning) (Figure 1). The post feeding larvae and pupae of *C. megacephala* were observed on day-5 (5 p.m.) and day-11 (11 a.m.) in the forest habitat, respectively. The same were found on day-6 (11 a.m. – 3 p.m.) and day-12 (9 a.m.) in the Mount Kapur Cave, correspondingly. Apart from the first-second and second-third larval stages, other stages of life cycle for *C. megacephala* did not exhibit statistical differences between the two habitats (Table 8, $p > 0.05$). Similarly, statistical difference also did not prevail for the total duration (240-260 hours, about 10-11 days) for completing the life cycle between the two habitats (Table 9, $p > 0.05$). The durations fell well within the reported durations for *C. megacephala* reported from field experiments in Peninsular Malaysia (Mahat *et al.*, 2009; 2014; 2016; Denis *et al.*, 2018).

In this present research, the predatory and cannibalistic behavior of *C. villeneuvi* was notably evident during the second and third instar larval stages; the same for *C. rufifacies* was observed during the third instar larval stage alone. Such behavior has also been reported by previous researchers in Peninsular Malaysia (Omar *et al.*, 1994b; Mahat and Jayaprakash, 2013). These authors indicated about the predatory behavior of the third instar larvae of *C. villeneuvi* and *C. rufifacies*, attributing the behavior to their larger larval size than the prey. In this context, (a) the predatory behavior of the second instar larvae of *C. villeneuvi*, as well as (b) the ability of the smaller larvae *viz. C. villeneuvi* and *C. rufifacies* to predate larger larvae e.g. *Hypopygiopsis* species observed here have never been reported so far. Because of this behavior, other larvae of necrophagous flies were observably moved away from the maggot masses of these two predatory species on the carcasses.

While the first, second and third instar larvae, as well as postfeeding larvae, pupae and tenerals for *C. villeneuvi* in the surrounding forest habitat were observed on day-2 (7-9 a.m.), day-2 (5 p.m.), day-3 (7-9 a.m.), day-8 (9 a.m.), day-9 (9 a.m.) and day-13 (9 a.m. – 5 p.m.), correspondingly, the same for the Mount Kapur Cave were noted on day-3 (7-9 a.m.), day-4 (11 a.m.), day-5 (11 a.m. – 3 p.m.), day-8 (9 a.m. – 5 p.m.), day-9 (9 a.m. – 5 p.m.) and day-15 (9 a.m. – 5 p.m.), respectively (Table 6). Significantly longer periods of eggs-first instar, first-second instar, second-third instar larvae, as well as postfeeding larvae-pupae, and pupae-tenerals of *C. villeneuvi* that infested carcasses in the Mount Kapur Cave were observed when compared with those in the surrounding forest habitat (Table 6, $p < 0.05$). However, significantly longer period of third instar-postfeeding larvae of this species was found in the surrounding forest than that of Mount Kapur Cave (Table 10, $p < 0.05$). Prolongation in developmental stages of *C. villeneuvi* observed in the Mount Kapur Cave can probably be associated with the combinatory effect of slightly lower ambient temperature, substantially lower light intensity and relatively higher percentage humidity in the habitat versus that of the surrounding forest habitat. The fact that such combinatory effect on the growth pattern of

necrophagous insects has never been reported in the literature, undertaking further endeavor to elucidate this aspect may prove to be an interesting forensically related study. The significantly longer period of third instar-post feeding larvae of *C. villeneuvi* in the surrounding forest habitat can be explained by the fact that the soil at that decomposition site was consistently soaked with water during the second and third day of decomposition, attributable to the presence of rain (Figure 1). While field studies (Omar *et al.*, 1994a; 1994b; Silahuddin *et al.*, 2015) that reported about *C. villeneuvi* in Peninsular Malaysia do not explicitly tabulate its complete life cycle, Mohd Salleh *et al.* (2014) attempted to provide such data *via* their laboratory-controlled experiment. They reported that *C. villeneuvi* required 9.00 ± 0.07, 9.34 ± 0.04 and 9.40 ± 0.02 days to complete the life cycle when reared at constant temperatures of 30, 27 and 25°C, respectively, with percentage humidity ranged between 70-85%. As for this present research, the total durations for completing the life cycle in Mount Kapur Cave (310.00 ± 3.46 hours, about 13 days) and its surrounding forest habitat (290.67 ± 12.20 hours, about 12 days) were statistically insignificant (Table 10, $p > 0.05$). In this context, the longer durations for completing the life cycle for *C. villeneuvi* observed in this present research may largely be due to fluctuations in the environmental conditions (especially ambient temperature as well as rain and humidity) at the decomposition sites.

As for *C. rufifacies*, its first, second and third instar larvae, as well as postfeeding larvae, pupae and tenerals in the surrounding forest habitat were observed on day-2 (9 a.m.), day-2 (5 p.m.), day-3 (7-9 a.m.), day-4 (1-3 p.m.), day-8 (5 p.m.) and day-12 (9 a.m.), respectively (Table 7). The same for Mount Kapur Cave were: day-3 (9 a.m.), day-3 (5 p.m.), day-4 (7-9 a.m.), day-5 (3-5 p.m.), day-9 (5 p.m.) and day-13 (5 p.m.), correspondingly (Table 7). The result revealed significantly longer periods of third instar-postfeeding larvae and pupae-tenerals for *C. rufifacies* in Mount Kapur Cave when compared with that of surrounding forest habitat (Table 10, $p < 0.05$). The prolongation in the periods observed here can be attributable to the same reason as discussed for *C. villeneuvi*. The total duration for completing life cycle in Mount Kapur Cave (median: 298.0, range: 296.0-298.0 hours, equivalent to 12.4 days) was significantly longer when compared with that of the surrounding forest habitat (median: 266.0, range: 264.0-266.0 hours, equivalent to 11.1 days). It appears that the total duration for completing the life cycle for *C. rufifacies* in the surrounding habitat was consistent with the findings reported by Mahat *et al.* (2016) in Kubang Kerian, Kelantan. As for the cave habitat, suitable comparison could not be made due to unavailability of the information in the body of literature.

For *Hem. ligurriens*, the first, second and third instar larvae, as well as postfeeding larvae, pupae and tenerals in Mount Kapur Cave were observed on day-2 (5 p.m.), day-3 (7 a.m.), day-3 (5 p.m.), day-5 (1 p.m.), day-7 (9 a.m.) and day-23 (11 a.m.), respectively (Table 8). Considering that this necrophagous species was not found in the surrounding forest habitat, statistical comparison of the life cycle between the two habitats cannot be made. Despite being indicated as one of the important necrophagous flies in forensic entomology, review of literature reveals no comprehensive data pertaining to the complete life cycle of *Hem. ligurriens* in Malaysia. The only available studies (Omar *et al.*, 1994a; Silahuddin *et al.*, 2015) were of field observations without explicitly providing the complete life cycles. In this regard Yang *et al.* (2015) reported a pioneering laboratory research that tabulated the complete life cycle of *Hem. ligurriens* at controlled temperatures. It can be made up from their Table 1 that *Hem. ligurriens* required 566.9 hours (equivalent to 23.62 days) to complete its life cycle at the constant temperature of 28°C and relative humidity of 70%. In this present research the completion of life cycle for *Hem. ligurriens* in Mount Kapur Cave occurred at 532.0 hours (equivalent to 22.2 days), about a day shorter than that of Yang *et al.* (2015). Such a shorter total duration observed here, when compared with that reported by Yang *et al.* (2015), can

be attributed to the fact that (a) the decomposition took place in a cave habitat with limited amount of light intensity, as well as (b) fluctuation and relatively lower ambient temperatures (min: 26.41 ± 0.99! and max: 27.15 ± 0.66!) at the decomposition site than that reported by Yang *et al.* (2015).

It has to be acknowledged here that, besides the species discussed above, other necrophagous species *viz*. *Hemipyrellia tagaliana* (Bigot), *Chrysomya chani* Kurahashi, *Chrysomya pinguis* (Walker), *Chrysomya nigripes* Aubertin, *Ophyra spinigera* Stein and *Ophyra chalcogaster* (Widermann) were also observed infesting the carcasses. However, due to logistic problems as well as their limited quantities and dispersive behavior, they were either found as larvae, pupae or tenerals alone. Therefore, proper discussion on their complete life cycles could not be made, necessitating further studies focusing on these necrophagous species at the same decomposition sites.

CONCLUSION

Investigation of the species of necrophagous flies in both Mount Kapur Cave and its surrounding forest habitat revealed the infestation by 13 different taxa of necrophagous flies on the decomposing rabbit carcasses *viz.* four species of Calliphorinae (Tribe Luciliini: *Hyp. violacea*, *Hyp. fumipennis*, *Hem. ligurriens* and *Hem. tagaliana*), six species of Chrysomyinae (*C. megacephala*, *C. villeneuvi*, *C. rufifacies*, *C. chani*, *C. pinguis* and *C. nigripes*), two species of Azelinae (Tribe Azelini: *O. spinigera* and *O. chalcogaster*) as well as an unidentified Sarcophagidae. Results revealed that (a) *Hyp. violacea* and *Hyp. fumipennis* were the two earlier necrophagous flies that oviposited in all carcasses in both habitats and (b) significant delay in oviposition, as well as longer durations for completing the life cycles in several necrophagous fly species were observed in Mount Kapur Cave when compared with those of surrounding forest habitat.

LIMITATION

Although morphological differences in aspect ratios, presence of fur, as well as weight would limit suitable discussions of the findings for human forensic, rabbit carcasses were used due to its easy handling, as well as having sufficient body size to support insect infestation (Bharti & Singh, 2003). Therefore, replicating the same experiment using pigs that are similar to human bodies would be necessary for providing better insights into rates of decomposition and patterns of fly colonization.

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