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Developmental Stages of *Hypopygiopsis violacea* (Family: Calliphoridae), a Forensically Important Blowfly on Rat Carcass

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ABSTRACT

A study on blowfly developmental stages to estimate the time of death (TOD) of small mammal had been conducted during a rainy season. During this study, fresh Muller's rat (*Sundamys muelleri*) carcasses were used as the host sample, and the developmental duration of every larval stage, decomposition stages of host animal, ambient temperature and relative humidity were recorded. *Hypopygiopsis violacea* (Family: Calliphoridae) was recorded to be the first blowfly visiting and ovipositing on the carcass after the carcass being deposited, while *Chrysomya megacephala* and *C. ruffacies* were recorded as the most dominant calliphorids present during the decomposition process. This study estimated that the time for calliphorids to complete their life cycle, from an egg to an adult was approximately twenty-three days, and the decomposition of *Sundamys muelleri* took about nine days. Useful information on succession and rate of development of blowfly could enhance the knowledge of the length of time elapsed since death in particular host animal.

Keywords: Blowflies, small mammal, time of death

INTRODUCTION

Blowflies (Diptera: Calliphoridae) are the most prevalent insect that can be found on the dead animals. These flies are commonly known as one of the important components that contribute to the decomposition process in the ecosystem. There are two methods for estimating time of death (TOD) or postmortem interval (PMI) which are based on the life stages of flies and the succession patterns of specific insects on the corpses. Blowflies are most commonly used in the study to estimate TOD (Smith, 1986). Animal carcass or human cadaver usually used as the host to provide food and development (Anderson, 2001). Blowflies will colonize dead body within a minute or second (Bharti & Singh, 2003). They are known to become the earliest colonizer in the faunal succession of human cadaver to estimate precise TOD (Grassberger & Reiter, 2001; Smith, 1986). *Chrysomya megacephala* (Fabricius) are commonly the first calliphorid species that can be found surrounding a dead human body (Lee *et al*., 2004; Thevan *et al*., 2010) and animal carcass (Omar *et al*., 1994), followed by *Chrysomya rufifacies* (Macquart). Therefore, they provide the accurate time to estimate the TOD.

Study of forensic entomology has already been used and applied during the last three decades and it had been largely documented by studies in several regions of the world for examples such as study that have been conducted by Gruner *et al*. (2007), Moura *et al*. (1997), and Slone and Gruner (2007). These studies constitute useful references in forensic entomology literature which they give different applications of medico-legal investigation most importantly in determining the seasonal and thermal effects towards the development of calliphorid. The techniques that were recently used in forensic entomology allow experts in the field to collect strong entomological evidence and provide very useful information in collecting the evidence for a death investigation including TOD, geographical location of death and the season when death occur (Byrne *et al*., 1995).

There are commonly two main factors affecting the accuracy of TOD. First, the duration elapsed between death with the initial ovipositor and the duration of development of

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larval stage on an insect (Anderson, 2001; Greenberg & Kunich, 2002). Second, the development of calliphorids in different climatic condition which is mainly caused by high temperature range and rain (Mahat *et al*., 2009).

In Malaysia, the first study on larval growth parameter and growth rate of a forensically important blowfly, *Hypopygiopsis violacea*, was conducted by Chen *et al*. (2011). Blowflies from different species has different growth rate, and is also very dependent on the surrounding weather, which include indoor and outdoor environment (Thevan *et al*., 2010), which make it directly affected by the environmental conditions–ambient temperature and humidity, and the heat generated by maggots' aggregation (Slone & Gruner, 2007). As the temperature increases the development of larva will also increase and this is crucial in the process of larval development.

This study was carried out to investigate and record the duration of each developmental stage of blowflies, the time since the fly first laid eggs on the carcass, and the time taken for the complete development of the blowflies. This study determined the relationship between TOD and the development of larval stage of blowflies.

MATERIALS & METHODS

Preparation of live larval specimens

A total of six experimental replicates were conducted during rainy season, from October to December 2014. The average weights of rats were ranging from 255.0 to 270.0 gram. *Sundamys muelleri* was chosen as the sample organism. They are known to be abundant in many range of habitats and not listed in the protected species in Malaysian Borneo. The carcasses were placed in a separate cage and deposited at open space of the same location to attract insect to them. The insects were allowed to lay their eggs on the carcass naturally. The first group of flies that were attracted to the carcass was collected by using insect net for further identification. The observation was carried out for 15 minutes for every carcass. The eggs on the carcass were collected by using insect forceps and transferred into a rearing container which contained a bit of carcass flesh as larval food source. Chicken liver was used as the food source after the carcasses were completely decomposed and consumed by the larvae. The observation on the larval was done until the pre-pupa stage. Observation on the larvae was done twice daily (morning and late evening). Once it reached the pre-pupa stage, the larvae were transferred into a new container containing a saw dust. The observation continued until the emergence of adult fly. The time taken for every larval stage of flies was recorded based on the study conducted by Chen *et al*. (2011). The humidity and the temperature of experimental site were recorded.

Insect preservation and identification process

The emerged adult flies were preserved in 70% ethyl alcohol for further identification purposes. The identification was done by using voucher specimen from Zoology Museum, UNIMAS and the identification keys provided by Kurahashi *et al*. (1997). The identification of small mammal was done base on the description by Payne *et al*. (1985).

RESULTS & DISCUSSION

Development of the larval stage

There are many species of blowflies that are attracted to the dead bodies of animals and humans. In present study, *H. violacea* was the first blowfly species that had been observed visiting the rat's carcasses. A study conducted by Chen *et al*. (2008), also stated that *Hypopygiopsis* sp. was the first calliphorid observed to arrive on the monkey carcass. Omar *et al*. (1994) and Chen (2008) emphasized that *H. violacea* is an important blowfly species in forensic investigation since this calliphorid is known to be the first fly to arrive to a dead body and carcass.

In this study, it was observed that the adult of *H. violacea* first arrived on the rat's carcasses after 20 minutes the carcass was laid at the experimental site. Based on the observation, the adult blowflies started to feed on carcass' body especially blood. This result is strongly supported by Greenberg and Kunich (2002) who suggested the adults will feed on any secretions, including blood. While the gravid females will rapidly lay their eggs on the carcass. Based on this study, it was observed

that the oviposition of *H. violacea* took after 45 minutes. Table 1 shows the duration of larval stage development of *H. violacea*. During this study, it was observed that the *H. violacea* was able to lay about 50 to 100 eggs per female. Once the eggs were oviposited, the period for eggs maturation took about seven hours before the instar emerged. In the feeding phase, the larvae of this species had gone through the first (L1), second (L2), and early feeding stages (L3) with a total duration of 80.0±11.5 hours. This is in contrast with Chen *et al*. (2008), which recorded the total feeding phase (L1-early L3) of *H. violacea* is 50.0±2.0 hours.

Figure 1 shows the complete life cycle of *H. violacea.* It is estimated from our observation that the oviposition of *H. violacea* took around 45 minutes after the carcasses were left on site. This is strongly supported by Smith (1986) that mentioned the oviposition of blowflies could occur within a few minutes after death. Eggs laid by the blowflies were important for determination of the time since death. Therefore, the first and earliest insect that colonized on the carcass must be known to estimate the accurate time of death based on its life cycle (Joseph *et al*., 2011).

Based on our result, the time for egg of *H. violacea* to hatch was seven hours (Table 1). This finding was similar to the study by Chen *et al*. (2011), where the eggs of *H. violacea* hatched within 24 hours and the larvae began to feed on the food resource. After the egg hatched, the larvae started to feed on tissues of carcass. The growth rate *of H. violacea* increased significantly in feeding phase. Once fully grown, the larvae of *H. violacea* moved away from the food source for pupation. The post-feeding larvae migrated away from the body to pupate, although some species pupated on, or in the immediate vicinity of the body (Greenberg & Kunich, 2002).

It was recorded that the duration of pupal stage was taking a long period of time which was 216.00±0.00 hours (Table 1). However, in some species of blowflies, they tend to spend about 50% of their juvenile development during the pupal stage (Sharma *et al*., 2015). This may be due to the preparation of the pupa itself in their development before they emerge as adults, thus took longer time to complete their life cycle.

Table 1. The duration of *Hypopygiopsis violacea* development.

Stages	**Duration**	
	Mean ± SE, hours	Day and hour
Egg	7.0 ± 0.0	7 hours
First instar, L1	15.0 ± 0.0	15 hours
Second instar, L2	22.0 ± 0.0	22 hours
Third instar, L3:		
Early L3: Feeding phase	48.0 ± 0.0	2 days
Late L3: Post-feeding phase	240.0 ± 0.0	10 days
Total feeding phase: L1 – early L3	80.0±11.5	2 days and 20 hours to 3 days and 20 hours
Total larval period: L1 – L3	320.0±6.15	13 days and 2 hours to 13 days and 14 hours
Pupal	216.0±0.0	9 days
Adult fly	536.0±6.15	22 days and 2 hours to 22 days and 14 hours

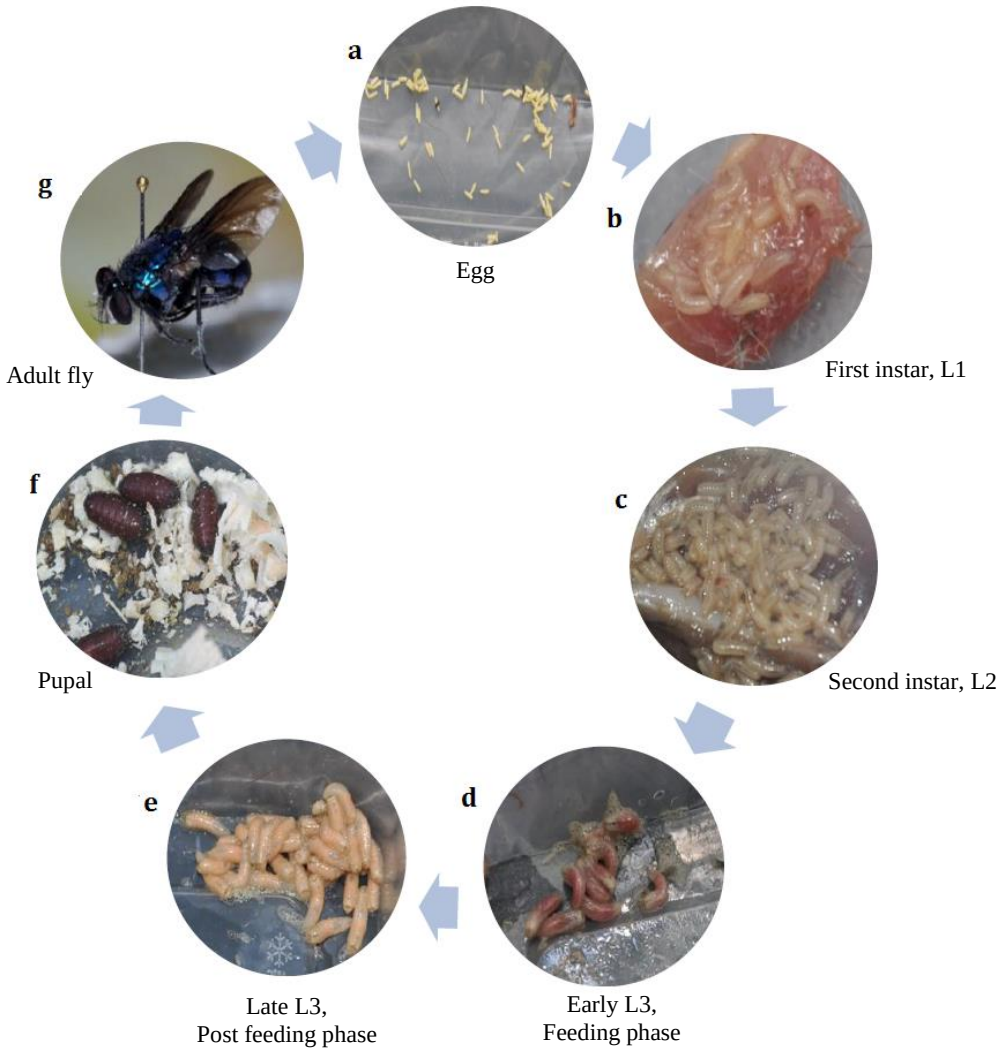

Figure 1 (a-g). The life cycle of *Hypopygiopsis violacea*.

Humidity on blowflies development

Table 2 shows the mean and standard deviation of ambient temperature and humidity during the development of each stages of *H. violacea*. The mean and standard deviation for adult stage was not recorded since it had emerged on the early morning at 9.00 am.

The rate of development of larval growth of blowflies depends on its body and surrounding temperature. Thus, their growths were directly affected by the changes in environmental conditions and the heat generated by maggot aggregations (Slone & Gruner, 2007). Chen *et al*. (2008) studied the growth rates of *H. violacea*, and reported that the development of egg took place within 6.25±2.25 hours with a temperature of 28±2ºC. This was quite similar to this study, where the average minimum and maximum ambient temperature of 30.5±0.32ºC and 30.8±0.67ºC respectively produced an egg development of 7.00±0.00 hours. In addition, *Chen et al*. (2008) stated the process of hatching will be accelerated during warm weather. On the other hand, hatching will not take place or will be delayed by one or two days if the weather is cold.

Based on this study, the minimum ambient temperature (ºC) was recorded during early feeding phase (29.7±1.21) while the maximum was recorded during post feeding phase (31.6±2.77). The average minimum and maximum temperature for the pupal stage (ºC) were 31.2±1.93 and 31.4±1.92 respectively. Matoba and Terazawa (2008) stated that the

major factors affecting the development of insect are temperature and weather. This finding is similar to Mahat *et al*. (2009), who determined that the rain had influenced the pupation period of flies by one to 34 days. This present study shows that the temperature and humidity also had influenced the development of the larval stage of *H. violacea*.

The temperature larval mass was influenced by the size of the carcass, weight, and the surface area. The smaller carcasses will absorb more heat as compared to the larger one. The high temperature larval mass reduced the time needed for larval development, thus the development becomes faster (Slone & Gruner, 2007). This study found that the total time of *H. violacea* larval period (L1 – L3) is 320.00±6.15 hours. In addition, this study produced a pupation time of 216.00±0.00 hours, with adult emergence at 536.00±6.15 hours. The pupal stages of *H. violacea* in this study took a longer period to emerge as an adult as compared to that of Chen *et al*. (2008) who recorded the adult emergence of *H. violacea* at 308.25±8.25 hours. This might be due to the rainy season during the experiments were conducted.

Low temperature was proved to slow down the organic matter decay process, inhibiting bacterial proliferation and preserving corpse tissues longer. On the other hand, higher temperatures were able to speed up the decomposition processes increasing bacterial proliferation and also the number and type of carrion insects (Campobasso & Introna, 2001). Different area and localities might have different ranges of temperature. Other than that, factors that affect the decomposition rates included the age of corpse, constitution, cause of death, ventilation and humidity (Campobasso *et al*., 2001). The sizes of carcass also affect the decomposition process. In this study, the complete process of decomposition took about nine days. Martinez *et al*. (2007) used a pig carcass which took 83 days to complete the decomposition stages. This revealed that the size of carcass influence the duration of decomposition process.

Table 2. The mean and standard deviation of ambient temperature and humidity on the development of larval stage of *Hypopygiopsis violacea*.

Stages	**Ambient Temp. (°C)**		**Humidity (%)**	
	Min.	Max.	Min.	Max.
Egg	30.5±0.32	30.8±0.67	84.5±6.08	90.5±2.21
First instar, L1	30.5±0.06	30.6±0.1	74.9±10.36	76.5±10.14
Second instar, L2	30.2±0.01	32.01±0.02	72.03±8.31	75.2±9.51
Third intar, L3:				
Early L3: Feeding phase	29.7±1.21	30.8±1.04	84.13±3.63	86.36±3.08
Late L3: Post-feeding phase	31.2±2.58	31.6±2.77	77.5±12.1	80.2±11.86
Pupal	31.2±19.93	31.4±1.92	79.6±11.39	81.7±11.26

Decomposition of carcass

This experiment observed five different stages of decomposition of host animal carcasses (Table 3). It took approximately nine days to complete the decomposition stages with the duration as follows: fresh stage (1-2 days), bloated stage (3 days), active decay (4-5 days), advanced decay (6-8 days) and dry remains (9 days onwards).

Early stage. Based on the observation, there was no unpleasant odor during the earlier stage of rat carcass. The physical appearance of the carcass still looks fresh. The first insect that visited the carcass was ants (Hymenoptera: Formicidae). After that, the first blowfly of family Calliphoridae started to visit the carcass. This can be observed 20 minutes after we laid down the carcass. **Bloated stage.** At this stage, more flies and ants was observed visiting the carcass which were identified as *C. megacephala* and *C. rufifacies.* The finding is strongly supported by the previous study in Malaysia which was conducted by Lee *et al*. (2004) and Thevan *et al*. (2010) who stated that the main observed species were *C. rufifacies* and *C. megacephala.* The carcass started to produce unpleasant odor and started to bloat. The first instar larvae of flies can be

Table 3. Decomposition stages and duration.

Decomposition Stage	Duration (day)
Fresh	1-2
Bloated	3
Active	4-5
Advanced decay	6-8
Dry remains	9 onwards

observed on the carcass during this stage. They started to feed on the flesh of carcass. **Active decay stage**. At this stage, a strong odor was present. Maggots were observed feeding on the tissues of the carcass. Most parts of the body tissue had been consumed. The dominant flies that were observed were *C. megacephala* and *C. rufifacies.* During the **advanced decay**, the maggot started to leave the carcass. They migrated into the ground to pupate. Less odors were detected. **Dry remains**. At this final stage of decomposition, no odors were detected and there were no flies present. The only remains were bones and hairs.

The present study classified that the decomposition stages of animal carcass consist of five stages which are fresh, bloated, active, advanced decay and dry remains. Several studies on stages of decomposition in long tail macaques (Azwandi & Hassan, 2009) and pig carcass (Heo *et al*., 2007) also revealed the same stages of decomposition. The five stages classification was also used human cadavers, primate and rabbit carcass (Ahmad *et al*., 2011; Arnaldos *et al*., 2005; Bharti & Singh, 2003).

In contrast, study done by Lee and Marzuki (1993) on monkey carcass classified that the decomposition of carcass consist of four stages which are fresh, decay, dry and remain stage. It was also suggested by Omar *et al*. (1992) where they studied about the arthropod ecological succession for forensic indicator. They observed that the larvae of *H. violacea* were only present during the fresh and bloated stage. Chen *et al*. (2008) stated that the larvae of *H. violacea* usually left the food sources before having a long post feeding time to get ready for pupation stage.

CONCLUSION

Insects' colonization of decomposing remains can appear minutes after death and can persist long after a body is skeletonized. It was determined that the complete life cycle of *H. violacea* was between 22 days and 2 hours, and 22 days 14 hours. It was also determined that there are five decomposition stages which were fresh, bloated, active, advanced decay and dry remains. The complete the decomposition process of host animal took about nine days.

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