DEVELOPMENT OF FOUR SPECIES OF FORENSIC FLIES, ESTIMATION OF POST-MORTEM INTERVAL AND THEIR APPLICATION ON FORENSIC CASES IN NORTH PENINSULAR MALAYSIA

by

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LIST OF ABBREVIATIONS

PMI	Post-mortem Interval
ADD	Accumulated degree day
ADH	Accumulated degree hour
DT	Developmental time (in hours)
DH	Degree hours
ADT	Accumulated developmental time (in hours)
RMSE (s.d)	Root mean square error (standard deviation)
mm	milimeter
cm	centimeter
kg	kilogram
˚C	Celcius
H.	*Hemipyrellia*
C.	*Chrysomya*
S.	*Synthesiomyia*
h	hours
IMR	Institute for Medical Research of Malaysia
h	height
w	wide
l	length

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1. Kumara, T.K., Abu Hassan, A., Che Salmah, M.R. & Bhupinder, S. (2009). The infestation of *Dermestes ater* (De Geer) on a human corpse in Malaysia. *Tropical Biomedicine* **26(1)**:73-79. (impact factor 0.649)

2. Kumara, T.K., Abu Hassan, A., Che Salmah, M.R. & Bhupinder, S. (2009). Larval growth of the muscid fly, *Synthesiomyia nudiseta* (Wulp), a fly of forensic importance, in the indoor fluctuating temperatures of Malaysia. *Tropical Biomedicine* **26(2)**:200-205.(impact factor 0.649)

3. Kumara, T.K., R. Henry L. Henry Disney, Abu Hassan, A. (2010). First record of two species of Oriental scuttle flies (Diptera: Phoridae) from forensic cases. *Forensic Science International* **195**: 5-7. (impact factor: 2.104)

4. Kumara, T.K., Abu Hassan, A., Che Salmah, M.R. & Bhupinder, S. (2010). A report on the pupae of *Desmometopa* sp. (Diptera: Milichiidae) recovered from a human corpse in Malaysia. *Tropical Biomedicine* **27(1)**:131-133.(impact factor 0.649)

5. Kumara, T.K., Abu Hassan, A., Che Salmah, M.R. & Bhupinder, S. (2010). Growth of *Chrysomya megacephala* (Fabricius) maggots in morgue cooler- A case report. *J. Forensic Sciences* **55(6)**:1656-1658. (impact factor 1.524)

LIST OF CONFERENCES/SEMINARS/WORKSHOPS

1. 44th Annual Scientific Seminar Malaysian Society of Parasitology and Tropical Medicine.Oral presentation title : Forensic entomology in Northern Peninsular Malaysia on 4th – 5th March 2008 at Grand Season Hotel Kuala Lumpur.

2. Continuous Medical Education (CME). Oral presentation title: Forensic entomology : Case studies on 14 March 2008 at Ambulatory Care Centre Hall, Penang Hospital.

3. 2nd USM Penang International Postgraduate Convention. Oral presentation title : Composition of forensically significant arthropods collected from human bodies in Northern region of Peninsular Malaysia on 18-20 June 2008.

4. 7th Scientific Meeting of Allied Health Professionals. Oral presentation title : Forensic entomology in Northern Peninsular Malaysia on 29-30 July 2008 at Serdang Hospital, Selangor.

5. The 6th regional IMT-GT Uninet Conference. Oral presentation title : Application of forensic entomology methods in estimating postmortem inetrval in Northern Peninsular Malaysia on 28-30 August 2008 at The Gurney Resort Hotel & Residences, Penang.

6. Universitas Airlangga-Universiti Sains Malaysia. Second Colloborative Conference. Life Science: Synergy for Enhancenment of Quality of Life. 10-11th February 2009, Surabaya, Indonesia. Oral presentation: the role of insects in legal investigation.

7. First National Forensic Entomology Workshop. Institute for Medical Research, Kuala Lumpur. 11-12 March 2009.

8. 46th Annual Scientific Seminar Malaysian Society of Parasitology and Tropical Medicine. Oral presentation title : Developmental times of forensically important flies in Malaysia on 24th – 25th March 2010 at Grand Season Hotel Kuala Lumpur.

9. Seminar & Workshop on Scholarly Publishing. Bayview Beach Resort, Batu Ferringhi, Penang. 28-30 September 2010.

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PERKEMBANGAN EMPAT SPESIS LALAT FORENSIK, PENENTUAN JANGKA MASA PASCAKEMATIAN DAN APPLIKASINYA DALAM KES-KES FORENSIK DI UTARA SEMENANJUNG MALAYSIA

ABSTRAK

Kajian ini mengkaji hubungan lalat forensik dan mayat manusia dari Julai 2007 sehingga Julai 2010 di utara Semenanjung Malaysia. Larva dikutip dan dibawa ke makmal, di mana 50 % larva tersebut dibela dengan diberikan daging lembu secara *ad libitum* dan 50 % lagi larva dimatikan dengan air suam (52 ± 10º C) dan kemudiannya disimpan dalam larutan Kahle. Konformasi spesis dilakukan setelah larva menjadi dewasa. Frekuensi kejadian (FO) dan dominan (D) lalat forensik ditentukan untuk persekitaran dalaman dan luaran. Lalat dewasa *C. megacephla*, *C. rufifacies*, *Liosarcophaga dux* dan *S. nudiseta* dibela sebagai koloni dan perkembangan larva mereka ditentukan. Selepas itu, persamaan model ditentukan untuk setiap spesis menggunakan persamaan linear am (GLM). Berdasarkan perkembangan larva yang dikaji dan persamaan model, jangka masa pascakematian ditentukan untuk setiap kes forensik menggunakan min panjang larva, tahap perkembangan larva, jam darjah terkumpul (ADH) dan persamaan model.

Jumlah nombor lalat forensik yang dikumpul adalah 154 spesimen dengan 95 spesimen dikumpul dari 27 mayat persekitaran dalaman dan 59 spesimen dari kes persekitaran luaran. Kajian ini melaporkan rekod kes pertama tiga spesis dalam Malaysia (kumbang – *Dermestes ater*, *Dermestes maculatus* dan lalat – *Desmometopa* sp.) dan dua rekod pertama spesis di dunia (*Megaselia curtineura*

(Brues), *Megaselia spiracularis* Schmitz) dikutip dari mayat manusia. Untuk persekitaran dalaman terdapat empat famili dominan (Calliphoridae, Sarcophagidae, Muscidae and Phoridae) dengan famili Calliphoridae sebagai famili yang paling sering berlaku (FO) antara keempat-empat famili manakala untuk persekitaran luaran hanya satu famili dominan iaitu Calliphoridae. Masa perkembangan larva dari telur/instar pertama sehingga dewasa untuk *C. megacephala*, *C. rufifacies*, *Liosarcophaga dux* dan *S. nudiseta* masing-masing, adalah 218 ± 12 jam, 206 jam, 307 ± 3 jam, and 337 ± 4 jam. Kajian model mendapati ralat antara nilai sebenar dan nilai ramalan perkembangan larva dalam persamaan model adalah 0.5 – 24.2 %, 0.8 – 19.3 %, 6.2 – 26.3 %, dan 1.8 – 14.9 %, masing-masing bergantung kepada tahap perkembangan larva untuk *C. megacephala*, *C. rufifacies*, *Liosarcophaga dux* and *S. nudiseta*,. Perbezaan jangka masa pascakematian di antara keempat-empat kaedah adalah tidak signifikan ($F_{3,184}$ 2.36, p = 0.07). Walaubagaimanapun, kaedah jam darjah terkumpul (ADH) hanya boleh digunakan sekiranya rekod suhu yang dialami oleh larva diperolehi.

DEVELOPMENT OF FOUR SPECIES OF FORENSIC FLIES, ESTIMATION OF POST-MORTEM INTERVAL AND THEIR APPLICATION ON FORENSIC CASES IN NORTH PENINSULAR MALAYSIA

ABSTRACT

The current study explores the relation of the forensic insects and human corpses during the period July 2007 until July 2010 in North Peninsular Malaysia. The larvae were sampled and taken into laboratory, where 50 % of the larvae reared on beef meat *ad libitum* and the other 50 % killed in warm water (52 ± 10 ºC) and preserved in Kahle's solution. The species confirmations were done when the reared case maggots emerged. The frequency of occurrence (FO) and dominance (D) of forensic flies were calculated for indoor and outdoor environments. The adults of *C. megacephala*, *C. rufifacies*, *Liosarcophaga dux* and *S. nudiseta* were maintained as colony and their immature life cycle were established. Then, modeling equations was established for each species using general linear model (GLM). Based on the established life cycle and the models equations, the postmortem estimates were determined for each case using mean larval length, larval stage, accumulated degree hour (ADH) and modeling equations.

The total number of fly specimens sampled was 154 specimens with 95 specimens sampled from 27 indoor corpses and 59 specimens from 23 outdoor cases. Current study reported first case record of three species in Malaysia (beetles - *Dermestes ater*, *Dermestes maculatu*s and fly - *Desmometopa* sp.) and two first report species in the world (*Megaselia curtineura* (Brues), *Megaselia spiracularis* Schmitz) sampled from human corpses. For the indoor environment there are four dominant families (Calliphoridae, Sarcophagidae, Muscidae and Phoridae) with the

Calliphoridae was the most frequent family amongst the four families, whereas for outdoor environment only one dominant family which was Calliphoridae. The immature developmental time from eggs/first instar until emergence stages for *C. megacephala*, *C. rufifacies*, *Liosarcophaga dux* and *S. nudiseta* was 218 ± 12 hours, 206 hours, 307 ± 3 hours, and 337 ± 4 hours, respectively. Modelling study found the error between the actual and predicted accumulated developmental time in established models was 0.5 – 24.2 %, 0.8 – 19.3 %, 6.2 – 26.3 %, and 1.8 – 14.9 %, depending on the larval stages for *C. megacephala*, *C. rufifacies*, *Liosarcophaga dux* and *S. nudiseta*, respectively. The differences of PMI estimates among the four methods was not significant ($F_{3,184}$ 2.36, p = 0.07). However, ADH must only be used when the temperature records experience by the larvae was available.

CHAPTER 1

INTRODUCTION

1.1 Forensic entomology

Forensic entomology is probably one of the oldest branches of forensic sciences (Gupta & Setia, 2004). Researchers defined forensic entomology as the application of knowledge of insect biology to civil proceedings and criminal trials (Keh, 1985; Catts & Goff, 1992; Varatharajan & Sen, 2000; Turchetto & Vanin, 2004a; Eberhardt & Elliot, 2008; Hall, 2008; Matuszewski et al., 2008). Byrd & Castner (2010) defined forensic entomology as a broad field where arthropod science and the judicial system interact. Within this broad definition, they divide forensic entomology into three categories: urban, stored-product, and medico-legal (Byrd & Castner, 2010).

Urban forensic entomology concentrates mainly on controversies involving termites, cockroaches and other insect problems accruing to the human environment (Byrd, 2001; Hall, 2001). Stored-product forensic entomology generally deals with arthropods infestation or contamination of a wide range of commercial products (Catts & Goff, 1992). In both stored-product and urban entomology, the problems generally involve damage, need for an assessment of actions to assign blame, a solution, and, typically, some form of monetary adjustment (Goff, 2005).

On contrary, medico-legal forensic entomology, deals with arthropod involvement in events surrounding felonies, usually violent crimes such as murder, suicide and rape but also includes other violations such as physical abuse and contraband trafficking (Smith, 1986). Medico-criminal forensic entomology is used mainly to estimate the post-mortem interval or place of death based on the developmental rates and the succession ecology of specific insects that feed on carcasses (Catts & Goff, 1992; Wolff et al., 2001; Goff, 2005; Manlove & Disney, 2007; Hall, 2008).

Forensic entomology was established in Malaysia since 1950s (Reid, 1953). From year 1950 until year 2005, few publications relevant to forensic entomology have been published (Cheong et al., 1973a; Baharudin et al., 1994a; Baharudin et al., 1994c; Baharudin et al., 2003). Following year 2005 onwards, the research progressed with numerous first reports of species occurrences on carrions and human corpse (Heo et al., 2008b; Nazni et al., 2008; Kurahashi & Tan, 2009; Zuha et al., 2009), however none the studies explored the preferences of flies towards indoor and outdoor environment. Though there are few case specimen reviews (Lee, 1989 & 1996; Lee et al., 2004) those specimens were sampled by medical staffs and not an entomologist. Thus, the question arise whether the specimens were properly sampled? In addition, numerous studies on larval growth were done in Malaysia at constant temperature (Mohd. Iswadi et al., 2007; Chen et al., 2008; Ahmad Firdaus et al., 2009). Do the larval growths differ if it is grown under fluctuating room temperature? Then, when it comes to PMI estimations, different entomologist used different methods to estimate PMI. Were the outcomes of those PMI estimates differ

according to the methods used? These were the questions/justifications that were explored in current research.

1.2 Objectives

- To establish the composition of forensically important fly species in north Peninsular Malaysia.

- To establish the larval growth of *C. megacephla*, *C. rufifacies*, *Liosarcophaga dux* and *S. nudiseta* flies in north Peninsular Malaysia.

- To establish models for the larval growth of *C. megacephla*, *C. rufifacies*, *Liosarcophaga dux* and *S. nudiseta* flies in north Peninsular Malaysia.

- To determine the post-mortem interval using accumulated degree hours (ADH), mean larval length, larval stages and modelling equation.

1.3 Research hypothesis

- To observe the preference of forensic fly species towards indoor and outdoor human corpses.

- To prove that the larval developmental time of *C. megacephala*, *C. rufifacies*, *Liosarcophaga dux* and *S. nudiseta* were different in fluctuating indoor temperature.

- To prove that the larval developmental time of *C. megacephala*, *C. rufifacies*, *Liosarcophaga dux* and *S. nudiseta* can be predicted using larval stages and mean larval length.

- To compare the differences of PMI estimates using accumulated degree hours (ADH), mean larval length, larval stages and modelling equations.

CHAPTER 2

LITERATURE REVIEW

2.1 History of forensic entomology

Insects were first used in a forensic context in 13th century in China (Amendt et al., 2004). This was the first documented medico-criminal forensic entomology case (Benecke, 2001). The case was about a local death investigator, who determined that the presence of adult flies on the washed sickle (which used to kill the victim) confirm the guilt of a murder suspect, who subsequently confessed to the crime (Adair, 2001). The case was documented in a book called "Washing aways of wrongs" written by Sung Tz'u, explaining the importance for insects in crime scene investigations (Goff, 2000; Gennard, 2007).

In the mid 19th century, Orfila, a pathologist, listed 30 insects and other arthropods that visited a corpse to feed and oviposit (Greenberg, 1991). His observation may be the first to systematize knowledge of arthropod succession on a human corpse, but to Bergeret (in 1855) goes to the credit for applying this knowledge in an actual case (Greenberg, 1991; Benecke, 2001). In March, 1850, the mummified body of an infant was discovered in a house which has been rented by four families during the period of three years. Bergeret found large numbers of empty puparia in several cavities of the body which he determined as *Sarcophaga carnaria* (L.). Based on this entomological evidence, he concluded that the larviposition occurred in 1848 and exonerates three families (Greenberg & Kunich, 2002).

Though Bergeret first applied the entomological evidence in a case work it was Jean Pierre Megnin (in 1894) that regarded as the first person who undertook a scientific research on forensic entomology (Benecke, 2001; Gupta & Setia, 2004). Megnin, the founder of forensic entomology identified eight stages in the decomposition of a human in an exposed environment and the insects associated with each stage (Greenberg, 1991). He recorded the insects that are attracted to corpses for almost a couple of decades and compiled his findings in the form of a book titled *La faune des Cadavers* in 1894 (Gupta & Setia, 2004). In it, he expanded his former theory of four insect waves for freely exposed corpses to eight succession waves and for buried corpses, he reported two waves. He also described 19 cases, including his own between 1879 and 1888 (Gupta & Setia, 2004). Since then, the researchers in United States, Russia and Canada started to conduct studies on the carrion fauna respective of their geographical regions.

In Central Europe, Leclercq & Nuorteva, use entomological evidence in their case work, from 1960s to the 1980s (Benecke, 2001). In the late 19th century and early 20th century most of the PMI estimation is based on the succession pattern of the invertebrates on the human corpses and animals models.

In Malaysia, the first case involving medico-criminal forensic entomology was in 1950 from Penang. The case involving a woman whom had been shot by a bandit and the early 2nd instar of *C. megacephala* were collected from the body by a pathologist Dr. H.M. Nevin. In that situation, the PMI estimated between 16-24 hours prior the death of the women which corroborate with the police investigation

(Reid, 1953). The following case reported in Malaysia was in early 1972 where a male corpse was found near Rawang, Malaysia (Cheong et al., 1973a) where the PMI estimated was 5-6 days old. Since then, most of the entomological evidence in Malaysia was sent to Institute for Medical Research for PMI estimation.

2.2 Carrion invertebrates

Hundreds of arthropod species are attracted by corpses, primarily flies (Diptera), beetles (Coleoptera) and their larvae (Benecke, 2004). Forensic entomologist learned that insect growth, feeding, and migration are genus-specific and are as driven by temperature, daily and seasonally (Marks & Tersigni, 2005). Generally, among law enforcement practitioners, maggots and other insects have traditionally been regarded as unpalatable by products of the decomposition process (Adair, 2001).

According to Pujol-Luz et al. (2006) a corpse constitutes a dynamic system that supports a rich community of arthropods, which is affected by several local factors. Smith (1986) describes the invertebrate's occurring on carrion into four ecological categories as mentioned below. In general, the first three groups are most important for forensic purposes (Arnaldos et al., 2005). However, Amendt et al. (2004) mentioned for medico-criminal forensic entomology, the first two groups are most important which include species from order Diptera (flies) and Coleoptera (beetle).

a. Necrophages species - feeding on the carrion and the most important in establishing time of death. Many species in this category are of major significance during the early stages of decomposition.

b. Predators and parasites - feeding on other insects or arthropods. This group also contains schizophagous species, which feed on the carrion at first, but may become predaceous in later larval stage.

c. Omnivorous - species such as ants, wasps and some beetles that feed on both corpse and associated fauna.

d. Incidentals - arthropods that use the corpse as a concentrated resource extension of their normal habit such as springtails and spiders (which may become incidental predators) (Smith, 1986). This category is also termed as adventives. These are species with no real relationship to the decomposition body, but simply use it as an extension of their normal habitat (Goff, 2005).

2.3 Species identification

The common factor is that the quality of the evidence depends upon accurate identification and a thorough understanding of invertebrate biology (Gunn, 2006). In medico-criminal forensic entomology, species identifications and development of baseline data for all insect species found in human remains and/or at death scenes are crucial pieces of information that are required to conduct accurate future forensic analysis (Sukontason et al., 2006d). One problem facing the entomologist is the accurate identification of the maggots collected from a corpse (Catts & Goff, 1992).

All too frequently only dead specimens, often poorly preserved, are submitted for identification (Catts & Goff, 1992). Other researchers used the state of the art facilities such as scanning electron microscope (SEM) (Sukontason et al., 2003a; Sukontason et al., 2003d; Siriwattanarungsee et al., 2005) or DNA identification (Tarone & Foran, 2006; Pai et al., 2007; Wells et al., 2007; Harvey et al., 2008; Song et al., 2008; Wells & Stevens, 2008; Saigusa et al., 2009) for accurate identification of the species. These methods were billed as more advanced and scientific than morphological features (Gupta & Setia, 2004).

The identification based on the morphology of the insects can be done in two approaches. First, based on the taxonomic keys as were used in this research to identify the species or the second approach was based on the ultra structure for species identification using scanning electron microscope (SEM), which provides the greater details of species identification, especially in the first instar (Sukontason et

al., 2005d). Such studies of taxonomy using SEM in literature according to species were *Chrysomya nigripes* (Diptera: Calliphoridae) (Sukontason et al., 2005d), *Chrysomya megacephala* (Sukontason et al., 2003d), *Megaselia scalaris* (Loew) (Wolff & Liu, 1996; Sukontason et al., 2002; Sukontason et al., 2005a; Sukontason et al., 2005c), *Chrysomya rufifacies* and *Chrysomya villeneuvi* Patton (Sukontason et al., 2006a), *Sarcophaga* spp. (Sukontason et al., 2003a), *Chrysomya rufifacies* (Sukontason et al., 2003c), *Piophila casei* (Sukontason et al., 2001b); *Chrysomya bezziana* (Sukontason et al., 2006c), *Hemipyrellia ligurriens* (Wiedemann) (Sukontason et al., 2008b), *Chrysomya nigripes* (Sukontason et al., 2006b), and *Lucilia cuprina* and *Liosarcophaga dux* (Sukontason et al., 2006d).

2.4 Larval sampling and preservation

The preservation of maggots depends on the requirement of an entomologist. Researchers use varieties of killing and preservation methods such as, Pampel's solution (Tantawi et al., 1996; Turchetto et al., 2001), 70 % alcohol (Greenberg, 1991; Wells & Kurahashi, 1994; Aggrawal et al., 2003; Arnaldos et al., 2004; Klotzbach et al., 2004; Leccese, 2004; Turchetto & Vanin, 2004b; Pujol-Luz et al., 2006), hot water killed and 70 % alcohol (Benecke & Lessig, 2001; Lee et al., 2004), 75 % alcohol (Benecke, 1998; Anderson & Cervenka, 2002; Linnea, 2004), 80 % alcohol (Saukko & Knight, 2004; Sekar, 2004; Day & Wallman, 2006; Martinez et al., 2007).

In Thailand, Sukontason et al. (2008a) used hot water (~90 ºC) for three minutes to prevent larval shrinkage during the measurement procedure, fix their protein and prevent darkening of the specimens in 70 % alcohol. In Malaysia, Lee et al. (2004) reported in their three decades study, some of the specimens could not be identified because of poor killing and preservation techniques. According to Midgley & Villet (2008) it is recommended that insect larvae collected for forensic purposes should be killed using the same method as was used to create existing models for rate of development.

2.5 Important forensic flies in Malaysia

2.5.1 *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae)

Chrysomya megacephala (Fabricius) is a blowfly widely distributed over the Asian regions, South Africa, South America (Reigada & Godoy, 2005; Byrd & Castner, 2010). This fly is commonly known as oriental latrine fly. Although, *C. megacephala* has a pronounced activity peak during the heat of the afternoon, it is one of the first species to become active in the early morning hours and is one of the last species to depart carrion at nightfall (Byrd & Castner, 2010).

In Malaysia, *C. megacephala* was the dominant species at the Titiwangsa Range near Kuala Lumpur except for the highest altitude and exhibit synanthropic behaviour (Baharudin et al., 2003). The distribution of this fly is associated with the expansion of human movements and it has adapted very well with human environment that they can be found at almost every corner of earth, wherever human flourish mentioned (Wells, 1991; Baharudin et al., 2003). The larvae of this species are primarily carrion feeders, and the adult fly shows a preference for fresh remains(Byrd & Castner, 2010) .

2.5.2 *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae)

In Malaysia, *C. rufifacies* is one of the commonest species found on human corpse from different ecological habitats (Lee, 1996; Lee et al., 2004). The common name for this species is hairy maggot blow fly or hairy sheep maggot (Byrd & Castner, 2010). This species is mainly a tropical Australasian and Oriental fly with an altitude range from sea level to 1,250m, or 2,500m, depending on latitude and climate (Baumgartner, 1993; Tantawi & Greenberg, 1993a). It was reported to be introduced to the United States in 1980's and forensic entomologists have successfully monitored the spread of this predacious which is a cannibalistic species throughout most of United States (Tomberlin et al., 2006).

In Thailand, *C. rufifacies* was the primary species of fly found at death scenes involving exposed, burned, hanging or floating corpses (Sukontason et al., 2003c). It prefers large carcasses, such as rabbits, goats, sheep, kangaroos, and human cadavers over small one such as mice, rats, birds, lizards, and guinea pigs in field studies (Baumgartner, 1993). In North America, the adults was thought to cease its activity at 13ºC and the development of larvae at 15ºC (Baumgartner, 1993). However, recent study in temperate climate found that the adults of this species are active at temperatures as low as 9ºC and capable of developing below the lower threshold of 10ºC (Cammack & Nelder, 2010). Unlike *C. megacephala*, this species rarely enters dwellings and the larvae only develop on carrion, not excrement (Byrd & Castner, 2010).

2.5.3 Sarcophagids (Diptera: Sarcophagidae)

Flesh flies is a large family comprising of over 2000 species and representatives of this family are found throughout the world, with most species occurring either in tropical or warm temperate regions (Byrd & Castner, 2010). The eggs of this species hatch in the female fly's reproductive tract and therefore it lays first instar larvae (Gunn, 2006). In Malaysia, they have been known to cause myiasis (Cheong et al., 1973b). The viviparous females of sarcophagids are less fecund than blow flies and house flies and do not deposit all their larvae in the same carrion, rather distributing them evenly among several corpses (Galante, 2008).

The total life history strategy of sarcophagids is geared to the production of a few offsprings which rapidly utilize fresh carrion prior to exploitation and often overexploitation by the opportunistic calliphorids (Denno & Cothran, 1976). Due to difficulty in identifying the species of sarcophagids, Byrd & Castner (2010) suggested the larvae should always be reared to the adult stage to facilitate positive species identification. Recent study reported the molecular identification of 17 species of sarcophagids of forensic importance in Malaysia (Tan et al., 2010).

2.5.4 *Synthesiomyia nudiseta* (Wulp) (Diptera: Muscidae)

The fly *Synthesiomyia nudiseta* is one of the forensically important species in many countries such as in Brazil (Calderon-Arguedas et al., 2005a; de Souza et al., 2008), Egypt (Tantawi et al., 1996), southeastern United States (Lord, 1990), Thailand (Sukontason et al., 2007) and Malaysia (Baharudin et al., 1994a; Lee et al., 2004; Nazni et al., 2007; Kumara et al., 2009). This species can be found throughout the tropical and subtropical regions of the world (Byrd & Castner, 2010).

In Malaysia, the adults of *S. nudiseta* exhibit eusynanthropic character because it was only found near human premises (Nazni et al., 2007). The larvae are predacious and was noted to consume the larvae of *C. rufifacies* (Byrd & Castner, 2010). In Alexandria, Egypt it was a secondary invader of slow decaying carcasses in fall (Tantawi et al., 1996). The larvae are commonly found in animal and human faeces as well as in decaying vegetable materials, refuse, and garbage though they have been reported to prefer carrion as the food source of choice (Byrd & Castner, 2010).

2.5.5 *Chrysomya villeneuvi* Patton (Diptera: Calliphoridae)

The general morphology of this species resembled the larvae of *C. rufifacies*, except the tubercles of each body segment bearing sharp-ended spines that encircle the entire tubercle (Sukontason et al., 2003b). The second and third instar larvae are known to be predaceous on other dipteran larvae, even attacking predatory larvae such as *C. rufifacies* (Kurahashi & Chowanadisai, 2001). Adult habits are unknown, but it is not encountered indoors (Senior-White et al., 1940).

2.6 Fly life stages

The development of insects involves three major stages; the embryo, the immature and the adult. These insects either feed or develop on corpses at different stages of their development (Centeno et al., 2002). Their eggs vary greatly in appearance and are covered with a shell that varies in thickness, sculpturing and colour according to species (Triplehorn & Johnson, 2005). The embryonic development occurs within the eggs, which is well supplied with yolk and surrounded by a delicate outer shell or chorion. The flies undergo what is known as holometabolous, or complete metamorphosis.

After the eggs hatch, larvae develop will go through three distinct stages of growth that are termed instars (Happ, 1984; Denlinger & Zdarek, 1994; Marks & Tersigni, 2005). The larvae feeds and grows, molting several times followed by a wandering or post feeding stage, then prepupa and pupal stage until the adult

emerges (Happ, 1984; Adair, 2001). The immature and the adult stages are different in physical form and often live in different habitat and habit (Triplehorn & Johnson, 2005). Their larvae, devoid of legs and even lacking the conspicuous body divisions of head, thorax and abdomen, bears little resemblance to the adult (Happ, 1984; Denlinger & Zdarek, 1994).

The first instar feeds for a period of time, moults into a second instar, which again feeds, then molts into a third instar (Lord, 1990; Anderson & Cervenka, 2002; Centeno et al., 2002). The first instar stage is often the hardest to see when conducting a search for entomological evidence, much less evident than the eggs themselves (Haskell et al., 1997). The second instar is usually the shortest duration of any of the life stages for the blowflies, lasting sometimes no more than 8 to 12 hours at moderate temperature (Haskell et al., 1997). The larvae increased 10-fold in larval size in just a few days, doubling its size after each molt to accommodate the rapidly expanding crop and intestine, and all owes to the plasticity of the cuticle (Greenberg, 1991). At each of these moult, the insect sheds its outer layer, as well as its internal cephalopharyngeal skeleton or mouthparts and the lining of its tracheal system (Happ, 1984; Anderson & Cervenka, 2002). Therefore, each new stage has a new set of spiracular slits and a new set of mouthparts (Anderson & Cervenka, 2002). Breathing is performed through a pair of spiracles in the rear end of the body and a pair of anterior spiracles that appears after the first moult while the posterior spiracles, which located at the rear of the larvae were useful to determine the larval stage (Centeno et al., 2002).

Clarkson et al. (2005) suggested the larvae was considered to have reached a particular stage of development when approximately 10 % (visually estimated, as a precise count at those stages of development was impractical) of the population reach that stage. Typically, large numbers of larvae hatch together and move around the corpse as a group and by doing so, they disseminate bacteria and secrete enzymes which enable them to consume virtually all of the soft tissues of the corpse (Lord, 1990).

Larval growth takes several days to several weeks depending upon species, environmental conditions and the numbers of larvae present (Lord, 1990). During these feeding stages, the crop or also called food storage organ in actively feeding second instar and third instars is usually red or brown and clearly visible (Greenberg, 1991; Anderson & Cervenka, 2002). The crop colour changes from red to amber during the phase of rapid shrinkage and to a dark spot before pupariation (Greenberg, 1991). Normally, once a larva has reached a critical size and is committed to metamorphosis, though it still continues to feed. The period prior to attaining critical size is sometimes referred to as the obligatory feeding phase because it is absolutely essential for the onset of metamorphosis, while in the subsequent period of feeding, the facultative feeding phase, food consumption continues but is not essential for committing the larva to metamorphosis (Denlinger & Zdarek, 1994).

At the end of the facultative feeding period, a conspicuous switch in larval behaviour occurs (Lord, 1990); the larva ceases feeding and leaves the food and if presented with additional food, rejects it (Denlinger & Zdarek, 1994). This stage is

called as post feeding or wandering stage. After a period of time in post feeding stage, the larvae leave the body and search for a safe, protected area in which to pupate (Anderson & Cervenka, 2002). The process of larvae in post feeding stage turning into pupa called pupariation. Pupariation is a complex process of structural and morphological change that can occur at any time of the day. The first behavioural change of pupariation is a gradual immobilization of the wandering larva. Some species, for example flesh fly larvae tan precociously in the peritreme region (around the posterior spiracle) approximately two hours before immobilization (the red spiracle stage) and in some calliphorid larvae the cuticle changes from glossy to opaque pupae shortly before pupariation.

The next visible evidence of pupariation is retraction of the anterior segments bearing the cephalopharyngeal apparatus (mouthhooks). As a result of this action, the anterior spiracles, which are situated in the third apparent segment, assume a position at the front end of the body. When anterior retraction is completed, the larva shortens further and assumes a barrel shape (Denlinger & Zdarek, 1994). After a period of longitudinal contraction, the body surface becomes smooth as a result of cuticular shrinkage and forms a white puparium (Denlinger & Zdarek, 1994; Anderson & Cervenka, 2002). Its shape becomes stabilized by sclerotization, a process during which the newly restructured cuticle loses its plasticity and gradually hardens and darkens (Lord, 1990; Denlinger & Zdarek, 1994). The puparium is derived from the cuticle of the of 3rd instar, which hardens into a barrel-shaped brown coloured case (Centeno et al., 2002) and it is within this structure that the pupa is formed (Haskell et al., 1997).

Though the fly literature abounds with use of the term pupation to mean pupariation, these are two very distinct metamorphic events that are clearly segregated in time. Pupation frequently occurs many hours after pupariation. At the completion of pupariation, the fly is developmentally still a third instar larva, albeit a larva that is now immobile, contracted and has a cuticle that is heavily sclerotized and tanned. Because the larva during this phase of development does look so different from the wandering larva, the term prepupa is a useful designation (Greenberg, 1991; Denlinger & Zdarek, 1994).

In the pupal stage, within the puparium, the adult develops as old larval tissues and organs are remolded or replaced to form adult organs (Happ, 1984). When the sclerotization is completed, sequences of forensically useful developments unfold within the puparium. The prepupa stage last nine to ten hours at room temperature, then molts to become a cryptocephalic pupa and followed by phanerocephalic pupa (Greenberg, 1991). In this stage, the head is exerted, the thorax and respiratory horns are pushed posterior until each horn is beneath a delicate bubble membrane place dorsolaterally on segment four (Greenberg, 1991). Aided by pupal movement, the horns puncture the membranes and make contact with the exterior.

Metamorphosis proceeds and the next marker is pupal-adult apolysis. This begins in wing buds and legs and when completed, the pharate adult is enclosed in the transparent pupal exuvium (Greenberg, 1991). The fly must first force open the

operculum of the puparium, crawl out of the puparium and dig its way upward through the soil. Only after it is completely free of the substrate can it begin the process of wing and body expansion (Denlinger & Zdarek, 1994). A newly emerged fly is unmistakable with its wrinkled wings, tiny abdomen, pale color and a ptilinum that rhythmically inflates and deflates (Greenberg, 1991). After a few minutes to several hours, depending on the species, the wings expand and harden, the pigmentation develops and the insect is ready to fly (Triplehorn & Johnson, 2005).

2.7 Insect development and temperature

Insects are poikilothermic and therefore their rate of growth and development is largely dependant on the ambient temperature (Denlinger, 1978; Haskell et al., 1997; Briere et al., 1999; Honek, 1999; Higley & Haskell, 2001; Oliveira-Costa & Mello-Patiu., 2004; Gullan & Cranston, 2005). There are two terms used by researchers to note the development of the insects which is “development rate” and “growth rate”. Gilbert & Raworth (1996) defined “development rate” as the reciprocal of the time required to complete the egg, larval, or pupal stages while the “growth rate” is the larva’s rate of relative weight increase. The link between the insect development and the temperature was first reported by a French scientist named Reaumur in the eighteenth century (Higley & Haskell, 2001). However, the methods for using this understanding to predict insect development mostly dated from the 1950’s through to the present (Higley & Haskell, 2001).

Since the fly development is a temperature-driven event and varies according to species, accurate developmental data are fundamental to medico-criminal estimates (Haskell et al., 1997). When modelling temperature dependent development in poikilothermic insects, researchers can use either time or rate. Though, most entomologists prefer to use rate because many of the developmental rate models is derived from biochemical and biophysical principals, which rely on rates (Wagner et al., 1984; Kramer et al., 1991). From reviewed literatures, there are various models of predicting insect development and affect of temperature on the development of insect have been published (Stinner et al., 1974; Aliniazee, 1976; Allen, 1976; Logan et al., 1976; Pruess, 1983; Wagner et al., 1984; Higley et al., 1986; Allen, 1988; Kramer et al., 1991; Worner, 1992; Amoudi et al., 1994; Lactin et al., 1995; Gilbert & Raworth, 1996; Zuben et al., 1996; Briere et al., 1999; Honek, 1999; Ikemoto & Takai, 2000).

However, among those published literature, the degree day/hour approach is used widely because it requires minimal data for formulation, is easy to calculate and apply; often yields approximately correct values (Wagner et al., 1984) and have been successful in practical application (Worner, 1992). The insect development models in relation to temperature have been categorized into few basic approaches (Worner, 2008).

The oldest and most widely used model is the linear degree-day model, a simple linear description of insect development in relation to temperature (Briere et

al., 1999; Worner, 2008). This degree-day approach is widely used to understand development time in plant sciences, pest management and ecology and is useful in understanding insect and plant phenology (Higley et al., 1986; Charnov & Gillooly, 2003). Though this approach have been widely accepted, the model do not consider the factor such as biochemical reaction in the process of insect development, photo period or genetic factor, and fluctuating temperatures (Higley et al., 1986). In this model, the relationship between temperature and growth rate usually calculated as linear and one of the essential assumption in degree-day approach is that insect development is directly related to ambient temperature and time (Higley et al., 1986). The model has the advantage of simplicity and allows estimation of the developmental threshold and the degree day requirement of insect species (Briere et al., 1999) also have been widely used with linearity is found in almost 300 species examined so far (Gilbert & Raworth, 1996). Though, this model does not include nonlinearity at high and low temperatures and produces biased results at the lower and upper threshold limits (Briere et al., 1999).

The second approach to predicting insect phenology in relation to temperature encompasses the many non linear mathematical description used to describe non linear development of insects (Worner, 2008). Variety methods of non-linear have been found in literature (Stinner et al., 1974; Allen, 1976; Harcourt & Yee, 1982; Allen, 1988; Kramer et al., 1991). Though researchers admitted that for most species the degree-day approach is acceptable if the development occurs within the intermediate temperature (Allen, 1976; Higley et al., 1986; Kramer et al., 1991; Gilbert & Raworth, 1998; Lamb, 1998; Ikemoto & Takai, 2000; Charnov & Gillooly,

2003). The focus in this second approach is to predict the insect development at the low and upper threshold limit.

In estimating the PMI, it is the first approach frequently used by many workers (Catts & Haskell, 1990; Higley & Haskell, 2001; Gennard, 2007). The linear model forms the basis to the well known thermal summation or degree day approach to timing prediction (Worner, 2008). Degree days are defined as the number of degrees above a threshold temperature required for growth (Worner, 1992 & 2008) and degree days per day are accumulated over the time it takes the particular life stage to complete development (Worner, 2008). Hence, degree days or hours are the accumulated product of time and temperature between the developmental thresholds for each day (Amendt et al., 2004). Because this heat is accumulated as "thermal units", it can be calibrated and described as "degree-day" or "degree-hours", depending on the accuracy of temperature readings and time period involved (Haskell et al., 1997). Each development stage has its own total development requirement and each species requires a defined number of degree days to complete its development. This fact helps us to predict the time when a certain developmental stage will be reached (Amendt et al., 2004). The duration of development is calculated by adding up the number of thermals units (degree-hours or degree-day) contributed at each temperature (Wagner et al., 1984).

Some researchers called degree days as thermal units, or heat units or growing degree days (Worner, 2008). In this approach, the assumption that field temperatures lie within the more linear portion of the development rate function

suggests a linear model can be used for predictive purposes (Worner, 1992). This model, which assumes a linear relationship between development rate and temperature, is often accurate for intermediate temperatures, though it yields considerable error when temperature conditions tend toward the extremes temperature (Stinner et al., 1974; Nabity et al., 2006; Worner, 2008) because developmental rate becomes curvilinear at both high and low extremes with increases or decreases in temperature (Nabity et al., 2006).

The assumption in this models is, as the environmental temperature decreases, their rates of development slow and, if the temperature falls low enough, development will cease at their lower developmental threshold; as temperature increases, their rates of development increased up to a temperature optimum and they again decreased development at upper threshold temperature (Wagner et al., 1984; Trudgill et al., 2005).