Occurrences of Calliphoridae (Diptera) on Pig Carcasses in the Dry Season in Thailand

Nattida Rungsri[1,2], Sarit Suebpongsiri[1] & Taeng On Prommi[2]*
[1]Faculty of Forensic Science Royal Police Cadet Academy,
Nakhon Pathom Province, Thailand
[2]Faculty of Liberal Arts and Science, Kasetsart University, Kamphaeng Saen Campus,
Nakhon Pathom Province, Thailand

Abstract

Calliphorids are the first arthropods to colonize corpses; their composition and abundance vary according to location. They are used to estimate post-mortem intervals and surmise the relocation of corpses. This study was focused on Calliphoridae associated with pig carcasses in sheltered and unsheltered cages. Four domestic pig (*Sus scrofa* Linnaeus, 1758) carcasses were used to determine the Calliphoridae activity on carrion over a period of 30 days in the dry season (April 5 to May 4, 2016). Five decomposition stages were observed and a total of 23,440 Calliphoridae, belonging to 3 species [*Chrysomya megacephala* (Fabricius), *C. rufifacies* (Macquart) and *C. nigripes* (Aubertin)] were collected in this study. Decomposition was slightly faster on the carcass in unsheltered areas, as was the the number of Calliphoridae specimens, relative to sheltered carcasses. The relationship between environmental factors such as temperature in cages, relative humidity and the internal temperature of the carcasses (sheltered and unsheltered carcasses) were significantly correlated to the decompositions process and insect species ($P<0.01$). *Chrysomya megacephala* showed preference for a sunny area, whereas *C. rufifacies* preferred a shaded area. This study provides baseline information on the necrophilous fauna for estimating postmortem interval in cases of human death in Thailand.

Key words : Calliphoridae, Pig Carcasses, Sheltered Cage, Unsheltered Cage

* Corresponding Author
e-mail: faastop@ku.ac.th

Introduction

Decay of a dead body is greatly influenced by organisms that feed upon the body in the different stages of decomposition (Centeno et al., 2002; Nelder et al. 2009). The knowledge of this succession is an important tool in forensic studies to estimate the interval since death from the species of organisms found on the body (Centeno et al., 2002). The order Diptera, as early colonizing insects, is important in medicolegal case examination (Nelder et al. 2009). This can be useful in estimating the elapsed time since death or postmortem interval (PMI) (Catts & Goff, 1992). Insects have a particular ecological sequence for attacking cadavers, as Diptera are usually found in the initial stages of body decomposition (Gennard, 2007; Hitosugi et al. 2014), while Coleoptera such as dermestid, nitidulid and staphylinid beetles are late colonizers (Keshavarzi et al. 2015a; Keshavarzi et al. 2015b; Magni et al. 2015; Keshavarzi et al. 2015c). Among the sarcosaprophagous Diptera, the Calliphoridae are the initial and main consumers of carrion. They are considered the most important group of flies to the forensic entomologist, and they are used as indicators of the period of time since death of human cadavers (Shean et al. 1993; Zabala et al. 2014). Calliphoridae is a cosmopolitan group of calyptrate flies, comprising nearly 1500 recognized species worldwide (De Carvalho & Mello-Patiu 2008). In Thailand, there are 93 blow fly species belonging to 9 subfamilies including Ameniinae, Calliphoridae, Luciliinae, Phumosiinae, Polleniinae, Bengaliinae, Auchmeromyiinae, Chrysomyinae and Rhiniinae (Kurahashi & Bunchu, 2011). The composition and succession patterns of insects on carrion have been studied extensively in the northern part of Thailand, but there have only been a few studies conducted in the central part of Thailand, especially in the dry season. Hence, the aim of the present contribution is to investigate the duration of the decomposition stages, as well as the frequency and abundance of Calliphoridae associated with carcasses.

Materials and methods

1. Site description

The experiment was carried out in the Kamphaeng Saeng District, Nakhon Pathom Province, central Thailand (N 14°02.215′, E 099°57.818′) from April 5 to May 4, 2016. The study site was in a natural habitat inside the university. The area was surrounded by shrubs and trees, and there was a pond beside the study area. The site was selected to ensure limited public access to minimize potential human interference, allowing natural insects' association with pig carrion.

2. Experimental procedures

Four domestic pigs (*Sus scrofa* Linnaeus, 1758) were used as animal models to simulate human decomposition. For each experiment two iron cages, 100 cm x 100 cm x 100 cm, made with 2.5 cm x 2.5 cm wire meshing, were used to protect carcasses from vertebrate scavengers; one had a roof tile and the other one was roofless. Inside each cage, the body of a domestic pig weighing 18, 19, 23 or 25 kg was placed. Each individual pig carcass was put in separate plastic bag to avoid insect infestation before the setting up the experiment. The cages were separated by a distance of approximately 3 m. A distance of approximately 100 m separated the cages from each other.

Samples were collected twice a day (07.00-09.00 and 16.00-18.00) for ten days and thereafter once a day until the terminal dry remains stage. Sampling started on April 5, 2016 and ended on May 4, 2016. An aerial net for manual sampling was used to collect flying and crawling insects on the carcasses and the surrounding soil. The collected insects were preserved in 95% ethyl alcohol. During each visit to the cage, general weather (e.g. sun, cloudy, rain), physical appearance of the carcass, odours, and quantity and quality of the species present were recorded; photographs were taken and the calliphorids were collected from the corpses. Ambient temperatures and relative humidity were continuously registered with data loggers (24 records/day) placed in the cages, both in unsheltered and sheltered. Also, a thermometer was used to measure the corpse temperature.

According to the visible changes in the carcasses, the decomposition stages were classified as in Centeno et al. (2002): fresh, bloated, active decay, advanced decay and remains. The material collected was transported to the Faculty of Liberal Arts and Science, Kasetsart University, Kamphaeng Saen Campus. The specimens were identified to the species level using a taxonomic identification key (Sukontason et al. 2004; Sukontason & Sukontason, 2010). Specimens were all kept in 80% ethanol. The voucher material was deposited at the Faculty of Liberal Arts and Science, Kasetsart University, Kamphaeng Saen Campus, Thailand.

3. Data analysis

To compare the mean temperatures between the unsheltered and the sheltered site, a *t*- test was used to obtain a 95% confidence level. To evaluate the relationship between environmental factors of sheltered and unsheltered carcasses with decompositions process and Calliphoridae species, a Pearson correlation coefficient was used. Statistical analyses were performed using SPSS software (version 16.0).

Results

1. Climatic data

Average daily relative humidity and temperatures of carcass body surface in unsheltered and sheltered cages are shown in Fig. 1 and Fig. 2.

For the duration of the experiment, the mean temperature in the sheltered cage was 32.62±0.82°C. The mean temperature in the unsheltered cage was 33.87±1.18°C, which was significantly higher than the other. The mean temperature in pig carcasses in sheltered and unsheltered cages were not significantly different. The relative humidity was significantly different between the sheltered (70.87±4.75) and unsheltered cages (67.75± 4.46) (Table 1).

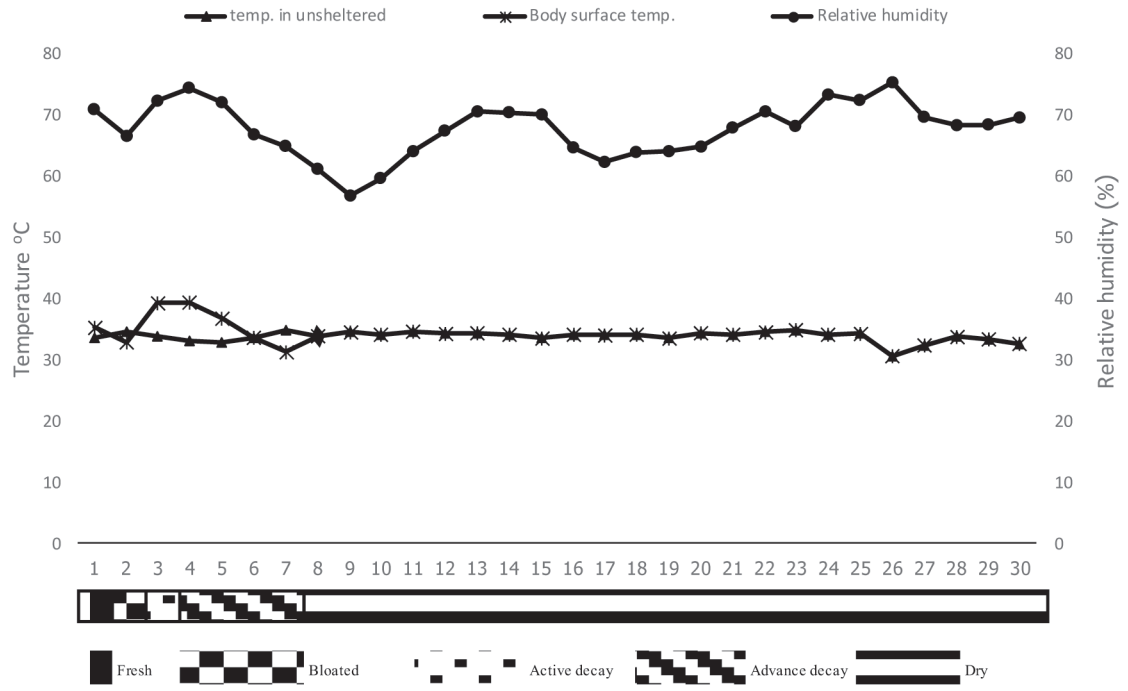


Fig. 1 Average temperature and relative humidity in unsheltered cage during 30 days

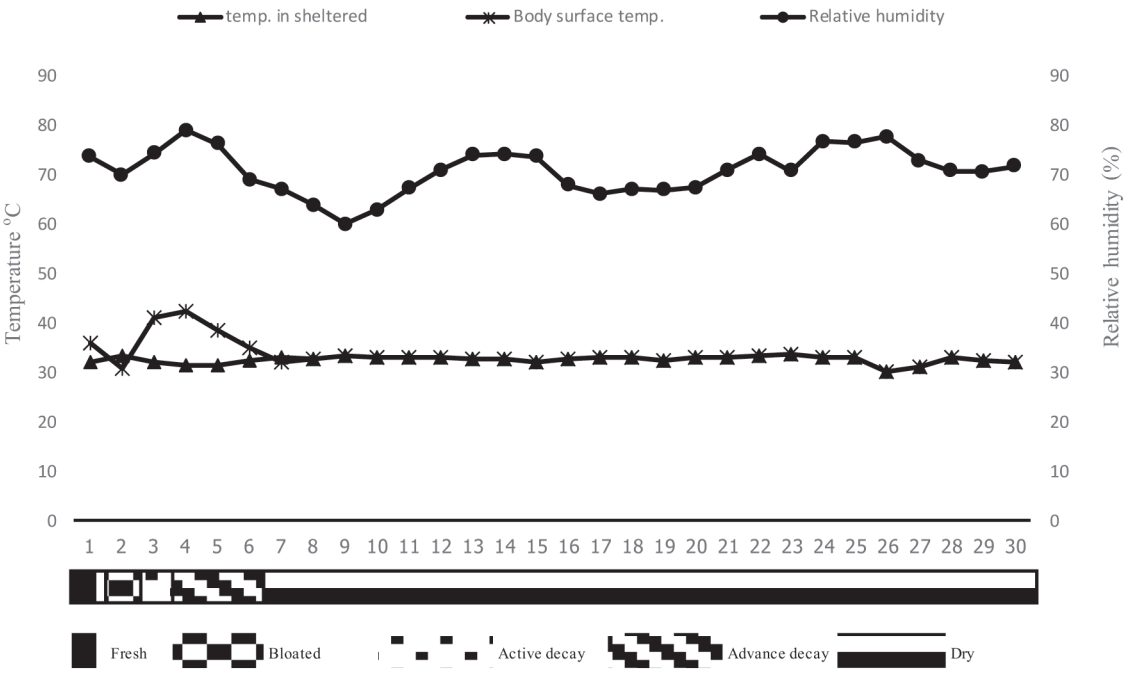


Fig. 2 Average temperature and relative humidity in sheltered cage during 30 days

Table 1 Mean±SD and minimum and maximum of environmental factors in sheltered carcasses and unsheltered carcasses during 30 days (5 April to 4 May 2016).

Factor	Sheltered cage	Unsheltered cage
Temperature in the cage (°C)	32.62±0.82*	33.87±1.18*
	(30.16-33.57)	(30.70-34.80)
Temperature in pig carcass (°C)	36.64±4.60	35.54±3.46
	(30.16-42.38)	(30.70-39.50)
Relative humidity (%)	70.87±4.75*	67.75±4.46*
	(60.02-79.00)	(56.88-75.30)

*significant at 95% (p<0.05)

2. Decomposition stages

Five decomposition stages were identified as described in Centeno et al. (2002): fresh stage, bloated stage, active decay stage, advanced decay stage, and dry/ remains stage (Fig. 3). The fresh stage began at the moment of death and lasted until day 1, both in unsheltered and sheltered cage, attracting 2 species (see Fig. 2 and Fig. 3). Adult blow flies of two species were collected during this stage: *Chrysomya megacephala* (Fabricius) and *Chrysomya rufifacies* (Macquart), and *C. nigripes* was only found in unsheltered cage. On day 2, bloating and odour were evident both in unsheltered and sheltered cages; odour and only a slight bloat was noticeable. This indicated that putrefaction had begun, so in both sites bloated stage was considered to begin on the 2nd day. The carcasses in both unsheltered and sheltered cages were very inflated on day 3 and had a very strong odour. Deflation of both carcasses due to Calliphoridae larvae breaking the skin started on day 4, representing the active decay stage. Calliphoridae larvae began to migrate massively from the carcasses on day 4 in both sites, marking the beginning of the advanced decay stage. The migration lasted approximately 3-4 days, with the number of larvae collected being higher in the sheltered cage. Biomass loss, evaluated visually, was much quicker in the carcasses exposed to the sun (unsheltered cage) than in the those in the shade (sheltered cage). The dry stage was reached on day 23 in the carcasses exposed to the sun (unsheltered cage), and on day 24 in the shaded carcasses (sheltered cage).

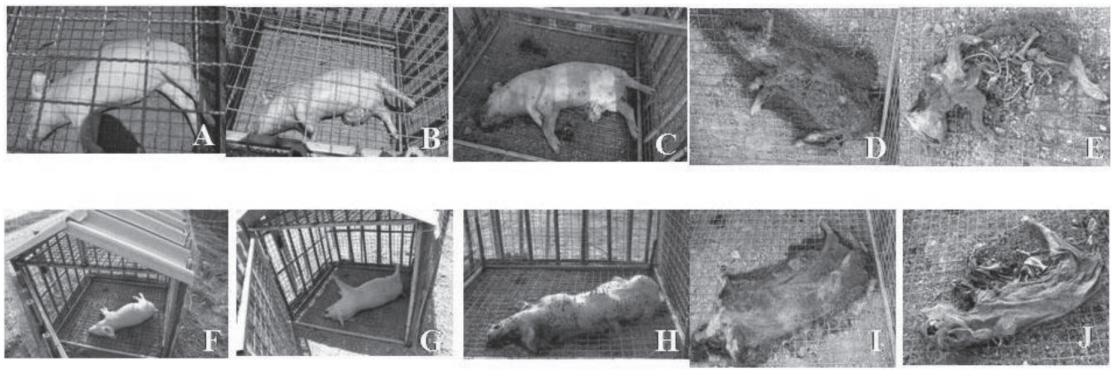


Fig. 3 Representative images of the 5 stages of decomposition observed within the study period (30 days) for both unsheltered (A-E) and sheltered (F-J) treatment groups: A,F=fresh stage; B,G=bloated stage; C,H=active decay stage; D,I=advanced decay stage; E,J=dry stage

3. Species composition

A total of 23,440 calliphorids were collected during the 30 days of the experiment. Of these, 11,678 specimens were captured in the sheltered site and 11,762 were collected in the unsheltered one (Table 2, Fig. 4 and Fig. 5). Three calliphorid species were identified: *Chrysomya megacephala* (Fabricius), *C. rufifacies* (Macquart) and *C. nigripes* (Aubertin) (Fig. 6). All the species were present both in unsheltered and sheltered sites. In general, a higher number of specimens were collected in the unsheltered site than in the sheltered one, particularly those from *C. megacephala.* The exception was *C. rufifacies,* which was slightly more abundant in the sheltered site. Occurrence of the species in the unsheltered and sheltered sites in the first 2 weeks is shown in Fig. 4 and Fig. 5. In both sites *C. megacephala* was the species most attracted to the corpses, followed by *C. rufifacies* and *C. nigripes,* the latter was present in very low numbers.

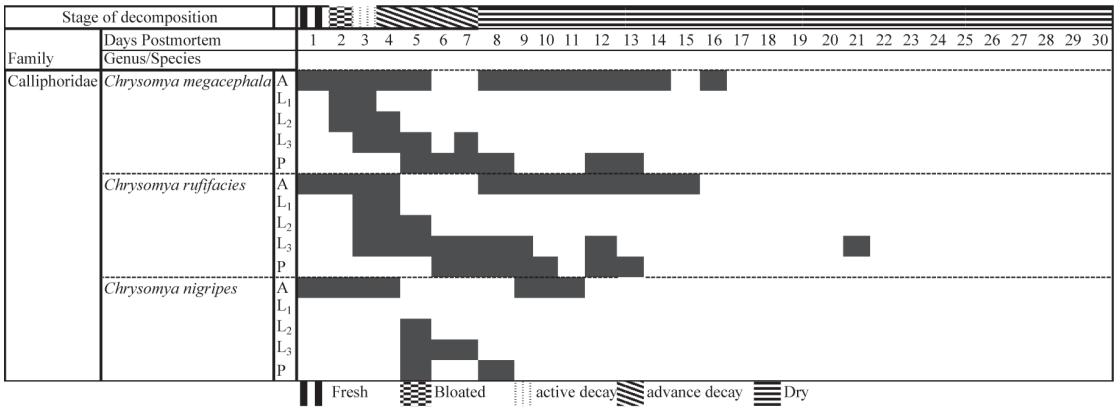


Fig. 4 The temporal succession pattern of Calliphoridae species on pig carcasses in unsheltered cages (n=2)

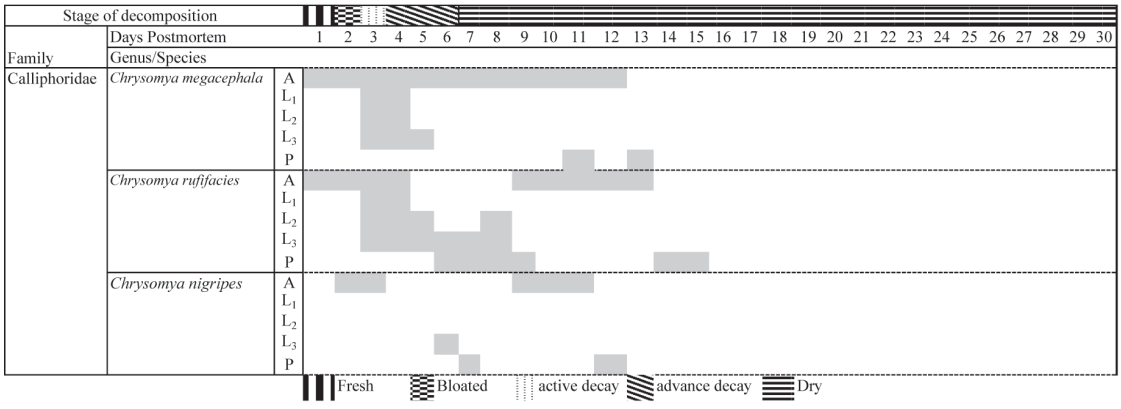
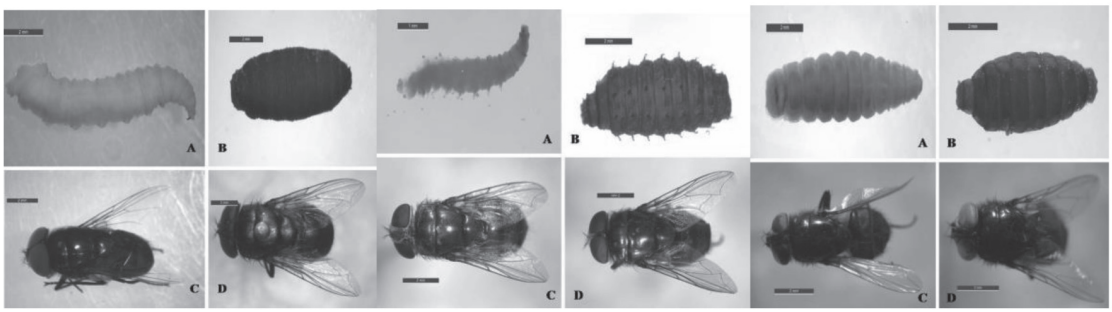


Fig. 5 The temporal succession pattern of Calliphoridae species on pig carcasses in sheltered cages (n=2)



A B C

Fig. 6 A; *Chrysmoya megacephala,* B; *C. rufifacies* and C; *C. nigripes:* A=larvae, B=pupae, C=female adult, D=male adult

Table 2 Abundance of Calliphoridae species (Diptera) collected in the sheltered and in the unsheltered carcasses; L_1 = first instar larval; L_2 = second instar larval; L_3 = third instar larval; P = Pupae; A = Adults)

Family	Genus/Species	Stage	Sheltered cage	Unsheltered cage
Calliphoridae	*Chrysomya megacephala* (Fabricius)	A	427	264
		L_1	1099	2496
		L_2	2683	3781
		L_3	1785	956
		P	208	7
	Total		**6202** (53.12%)	**7504** (63.79%)
	Chrysomya rufifacies (Macquart)	A	256	59
		L_1	146	55
		L_2	224	2186
		L_3	4246	1387
		P	383	21
	Total		**5255** (44.99%)	**3708** (31.53%)
	Chrysomya nigripes (Aubertin)	A	91	52
		L_1	0	0
		L_2	64	28
		L_3	54	133
		P	12	337
	Total		**221** (1.89 %)	**550** (4.68%)
Total			**11678 (100.00 %)**	**11762 (100.00 %)**

4. Statistical analysis

The two most abundant species (*C. megacephala* and *C. rufifacies*) corresponded to 98.11% and 95.32% of the total number of specimens captured in the first 2 weeks, respectively in the sheltered and in the unsheltered. They were analysed for the period of time that comprises the fresh, bloated, active and advanced decay stages. The there was a significant correlation between environmental factors such as temperature in cages, relative humidity and the internal temperature of the carcasses (sheltered and unsheltered carcasses) with the decompositions process and insect species ($p<0.01$).

Table 3 The results of the Pearson correlation test between environmental factors of sheltered and unsheltered carcasses with the decomposition process and Calliphoridae species

Factor/species	Temp. in cage	Relative humidity	Temp. in carcasses
Chrysomya megacephala	-0.094	0.272	0.554**
Chrysomya rufifacies	-0.484**	0.538**	0.789**
Chrysomya nigripes	0.165	-0.292	-0.130

**significant at 99% ($p<0.01$)

Discussion

Flies from the Diptera family Calliphoridae (blow flies) often are used to estimate the PMI since they usually represent the first wave of insect succession on cadavers (Ahmad et al. 2011). They land within minutes of death and oviposit within and around exposed areas such as the mouth, ears, nose and any open wounds, and therefore represent the most accurate entomological indicators of time of death.

The weather conditions during the study were recorded and were characteristic of the dry season in the region. These conditions contributed to the accelerated decomposition process (Rodriguez & Bass, 1983; Shean et al., 1993). According to the Pearson correlation test, the meteorological factors were associated with the Calliphoridae species specimen collected from the decomposing carcasses. This fact emphasizes that the carcass' temperature and temperature in cages are important with respect to the rate of carcass decomposition; relative humidity is also important because it acts directly on

the decomposition of the carcasses and promotes the emergence of an alternative decomposition stage (Moura et al., 1997). Because temperature and relative humidity conditions throughout the study exhibited little variation, it was not possible to demonstrate the influence of these factors on the presence of insects on the carcass or on the decomposition process itself.

The effect of unsheltered and sheltered conditions was analysed; slight differences in decomposition rates were observed between the four carcasses. Daily observation in the field suggested that the decomposition stages were progressing at different rates; they were much slower in the sheltered site. As an example of this, active decay was more advanced in the unsheltered carcass, similar to what was observed by Centeno et al. (2002). In the sheltered carcasses the swelling occurred slowly, with about a 2-day delay relative to the carcasses placed in the unsheltered site. A closer analysis of the characteristics and key moments that marked the progression from one stage of decomposition to another (odours, bloating, perforation of skin by larvae, migration and dryness) showed that they were simultaneous in the initial stages, in unsheltered and sheltered sites, but with differences occurring in later stages. The advanced decay stage lasted longer in the sheltered site, with the dry stage starting later than in the unsheltered site. The higher temperatures in the unsheltered site, due to direct sunlight, intensified the odour, attracting a large number of flies to oviposit on the corpse. The exposure of the growing larvae to the sun may have increased their metabolic rate, which accelerated the decomposition process and the apparently observed loss of biomass (Castro et al., 2011). The arrival of flies and oviposition started essentially at the same time in pig carcasses, but continued for a longer period of time on the sheltered carcass, due to the prolonged availability of suitable carrion (Shean et al., 1993).

Considerable differences between Calliphoridae succession dynamics in both sites were noticed. In the unsheltered site, Calliphoridae were the main and quicker flesh consumers, arriving in massive numbers in the first week, breeding on the cadaver and voraciously consuming it. In the sheltered site, the same species were present in the first week, but in lower numbers compared to the unsheltered site. As decomposition progressed, the opposite happened, with large numbers of specimen present in the sheltered site. The slower decomposition process in the unsheltered and the continuous availability of carrion allowed other families, as Muscidae, Sacrophagidae, among other

Diptera, to appear in very large numbers. In the unsheltered site, most of the other dipterans were less abundant, as dry stage was reached earlier and less carrion was left to consume (Centeno et al., 2002).

Despite the clear differences observed between unsheltered and sheltered conditions, there were no differences between them in regards to the number of Calliphoridae specimens attracted to the carcasses. In the first three decomposition stages, the carcasses exposed to the sun had higher numbers of specimen than in the shade. But an inverse trend occurred in the last decomposition stage, leading to a significant interaction effect between decomposition stage and insolation regime (Centeno et al., 2002).

In conclusion, this was a preliminary study; it is necessary to repeat and replicate it at different times of the year so as to provide multiple sets of baseline succession data for Thailand that encompass all seasons. However, the information obtained during this study could be useful for providing initial database information as no succession data was previously available in central Thailand. Furthermore, these results could also possibly stimulate other entomologists in Thailand and initiate future studies.

Acknowledgements

The authors would like to thank the Kasetsart University, Kamphaeng Saen Campus, for allowing the realization of the field work.

References

Ahmad, A., Ahmad, A.H., Dieng, H., Satho, T., Ahmad, H., Aziz, Al T., & Boots, M. (2011). Cadaver Wrapping and Arrival Performance of Adult Flies in an Oil Palm Plantation in Northern Peninsular Malaysia. *Journal of Medical Entomology,* 48(6), 1236-1246.

Castro, C.P., Sousa, J.P., Arnaldos, M.I., Gaspar, J., & García, M.D. (2011). Blowflies (Diptera: Calliphoridae) activity in sun exposed and shaded carrion in Portugal, *Annales de la Société entomologique de France (N.S.),* 47(1-2), 128-139.

Catts, E.P., & Goff, M.L. (1992). Forensic entomology in criminal investigations. *Annual Review of Entomology,* 37, 253-272.

Centeno, N., Maldonado, M., & Oliva, A. (2002). Seasonal patterns of arthropods occurring on sheltered and unsheltered pig carcasses in Buenos Aires Province (Argentina). *Forensic Science International,* 126, 63-70.

De Carvalho, C.J.B., & Mello-Patiu, C.A. (2008). Key to the adults of the most common forensic species of Diptera in South America. *Revista Brasileira de Entomologia,* 52, 390-406.

Gennard, D. (2007) *Forensic Entomology: An Introduction.* England: John Wiley & Sons.

Hitosugi, M., Kirinoki, M., Ishii, K., Morita, S., Furukawa, S., & Chigusa, Y. (2014). The importance of collecting live fly larvae from a severely decomposed human cadaver in winter: Case report. *Romanian Journal of Legal Medicine,* 22, 253-256.

Keshavarzi, D., Moemenbellah-Fard, M.D., Fereidooni, M., & Montazeri, M. (2015a). First report of Dermestes frischii Kugelann (Coleoptera: Dermestidae) on a human corpse, South of Iran. *International Journal of Forensic Science & Pathology,* 3(4), 113-115.

Keshavarzi, D., Moemenbellah-Fard, M.D., Fereidooni, M., Zarenezhad, M., & Fakoorziba, M.R. (2015b). New record of sap beetle, *Nitidula flavomaculata* Rossi (Coleoptera: Nitidulidae) on an outdoor mummified human corpse, South of Iran. *Journal of Entomology and Zoology Studies,* 3(3), 396-399.

Keshavarzi, D., Fereidouni, M., Moemenbellah-Fard, M.D., Nasiri, Z., Soltani, Z., Dabaghmanesh, T., & Montazeri, M. (2015c). Preliminary data on life cycle of *Creophilus maxillosus* Linnaeus (Coleoptera: Staphylinidae) and new report of this species on a human corpse, south of Iran. *International Journal of Forensic Science & Pathology,* 3(7), 144-147.

Kurahashi, H., & Bunchu, N. (2011). The blow flies recorded from Thailand, with the description of a new species of Isomyia Walker (Diptera, Calliphoridae). *Japanese Journal of Systematic Entomology,* 17(2), 217–278.

Magni, P.A., Voss, S.C., Testi, R., Borrini, M., & Dadour, I.R. (2015). A biological and procedural review of forensically significant *Dermestes* species (Coleoptera: Dermestidae). *Journal of Medical Entomology,* 52(5), 755-769.

Moura, M.O., Carvalho, C.J.B., & Monteiro-Filho, E.L.A. (1997). A preliminary analysis of insects of medico-legal importance in Curitiba, State of Paraná. *Memórias do Instituto Oswaldo Cruz,* 92, 269-274.

Nelder, M.P., McCreadie, J.W., & Major, C.S. (2009). Blow Flies Visiting Decaying Alligators: Is Succession Synchronous or Asynchronous? *Psyche,* 2009, 1-7.

Rodriguez, W.C., & Bass, W.M. (1983). Insect activity and its relationship to decay rates of human cadavers in east Tennessee. *Journal of Forensic Science,* 28, 423-432.

Shean, B.S., Messinger, L., & Papworth, M. (1993). Observations of diferential decomposition on sun exposed v. shaded pig carrion in coastal Washington State. *Journal of Forensic Science,* 38, 938-949.

Sukontason, K., & Sukontason, K.L. (2010). Flies of medical importance in Thailand. Chiang Mai: Chiang Mai Digital Work.

Sukontason, K., Sukontason, K.L., Ngern-Klun, R., Sripakdee, D., & Piangjai, S. (2004). Differentiation of the Third Instar of Forensically Important Fly Species in Thailand. *Annals of the Entomological Society of America,* 97(6), 1069-1075.

Zabala, J., Dĩaz, B., & Saloña Bordas, M.I. (2014). Seasonal Blowfly Distribution and Abundance in Fragmented Landscapes. Is It Useful in Forensic Inference about Where a Corpse Has Been Decaying? *PLOS ONE,* 9(6), e99668.

Authors

Miss Nattida Rungsri

Faculty of Forensic Science Royal Police Cadet Academy

Nakhon Pathom Province, Thailand

e-mail: nattida2257@hotmail.com

Asst.Prof.Pol.Lt.Col. Sarit Suebpongsiri

Faculty of Forensic Science Royal Police Cadet Academy

Nakhon Pathom Province, Thailand

e-mail: Saritsu2003@yahoo.com

Assist.Prof.Dr. Taeng On Prommi

Faculty of Liberal Arts and Science, Kasetsart University,

Kamphaeng Saen Campus

Nakhon Pathom Province, Thailand

e-mail: faastop@ku.ac.th