Journal of Medical Entomology, 54(5), 2017, 1157–1166
doi: 10.1093/jme/tjx082
Advance Access Publication Date: 21 April 2017
Research article

Direct Injury, Myiasis, Forensics

Variation in the Time of Colonization of Broiler Carcasses by Carrion Flies in Nakhonsawan Province, Thailand

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Subject Editor: Jason Byrd

Received 8 December 2016; Editorial decision 27 March 2017

Abstract

Carrion flies are the primary insects colonizing vertebrate carrion; however, limited information is available on the variation in the time of colonization (TOC) as related to time of placement (TOP) and time of death (TOD), particularly in Thailand. Three seasonal sets of nine broiler carcasses (euthanized and placed in field within 0.5 h after death) were placed in mesh enclosures within a disturbed deciduous dipterocarp forest at Nakhonsawan Province, upper-central Thailand, for 3 d to determine the colonization time by carrion flies. In total, 21,536 arthropods were collected using traps placed over each carcass. Carrion flies of the family Calliphoridae, Muscidae, and Sarcophagidae predominated (93.42%). Of these, *Chrysomya megacephala* (F.) (Diptera: Calliphoridae) and *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae) were the dominant species being 36.18% and 35.36%, respectively, across season. These species arrived immediately (5 min) after placement of the carrion in the field during the rainy season, while they were delayed 1–2 h during the dry season. *Chrysomya megacephala*, *C. rufifacies*, and *Parasarcophaga dux* (Thomson) colonized the remains. Time of colonization by *C. megacephala* and *C. rufifacies* occurred mostly at ∼1600–1700 hours (10–11 h after placement) for all seasons. In contrast, TOC by *P. dux* was delayed for 1 d during rainy and dry season. These results mark the first record of carrion fly colonization in this area and also may deserve important information for the further study as they demonstrate time of colonization differs from TOP and most importantly TOD.

Key words: colonization, initial decomposition, *Chrysomya megacephala*, *Chrysomya rufifacies*, *Parasarcophaga dux*

Medicocriminal entomology is the application of arthropods in forensic investigations. Historically, arthropod specimens were used for calculating the postmortem interval (PMI) or time since death (Anderson 1997, Turchetto et al. 2001, Oliveira-Costa and de Mello-Patiu 2004, Ying et al. 2013), cause of death (Magni et al. 2016), and supporting evidence of corpse relocation (Picard and Wells 2012). Information of the time of placement (TOP) as related to the time of colonization (TOC) and insect succession, in particular, is very important for calculating the PMI. Currently, little is known about the TOC and how it relates to the actual PMI of a corpse. As such, understanding the factors affecting decomposition and the associated arrival and departure of flies colonizing remains is a key to accuracy of entomological estimates of the PMI (Campobasso et al. 2001). This application is specifically true with regards to environmental factors impacting insect behavior, such as seasonal variation in environmental conditions (e.g., temperature and rainfall; Horenstein et al. 2012).

Carrion flies (Diptera) are generally attracted to corpse and carcass soon after they are made available. Byrd and Castner (2001) discuss a number of forensically important flies; however, three families in particular are known to be dominant; these families are the Calliphoridae (blow fly), Muscidae (muscid fly), and Sarcophagidae (flesh fly). For Thailand, Sukontason et al. (2007) reviewed 30 criminal cases from northern Thailand during 2000–2006. Thirty percent of cases were homicides, and remains were recovered from a forest setting. Eleven known species of Calliphoridae (*Chrysomya nigripes* Aubertin, 1932, *Chrysomya bezziana* (Villeneuve, 1914), *Chrysomya chani* Kurahashi, 1979, *Chrysomya megacephala* (F., 1794), *Chrysomya rufifacies* (Macquart, 1843), *Chrysomya villeneuvi* Patton, 1922, *Lucilia cuprina* (Wiedemann, 1830), and

Hemipyrellia ligurriens (Wiedemann, 1830)), Muscidae (*Hydrotaea spinigera* Stein, 1910 and *Synthesiomyia nudiseta* (van der Wulp, 1883)), and Sarcophagidae (*Liopygia ruficornis* F., 1794) were recorded as forensic important species of Thailand. Of these, *C. megacephala* and *C. rufifacies* were the two most common species found from all locations. These results were in accordance with cases reported from the northeastern region by Sritavanich et al. (2007, 2009). From a research perspective (Vitta et al. 2007, Sukchit et al. 2015), other fly species have been collected from pig carcasses as well. These species included *Chrysomya pinguis* (Walker, 1858), *Chrysomya thanomthani* Kurahashi and Tumrasvin, 1977, *Hypopygiopsis infumata* (Bigot, 1877), *Musca domestica* L., 1758, *Musca sorbens* (Wiedemann, 1830), *Boetcherisca peregrina* Robineau-Desvoidy, 1830, and *Parasarcophaga dux* Thomson, 1869 (Vitta et al. 2007, Sukchit et al. 2015). However, no experiments to date have focused on TOC and its relationship with TOP or the actual PMI of the remains. This study was conducted to determine TOC as related to TOP and time of death (TOD) by carrion fly species during the hot, rainy, and dry seasons of Thailand. The result of this study will provide the basic information of fly species attracted to carcass, and TOC from this study may be useful for forensic investigation in the future when estimating PMI of human remains.

Materials and Methods

Study Site

Experiments of fly colonization time were conducted in the open area of disturbed deciduous dipterocarp forest at Mahidol University, Nakhonsawan Campus, Phrayuhakiri district, Nakhonsawan province, upper-central Thailand. The location is 15.58189° N and 100.14640° E, which is 82.88 m above sea level (Fig. 1A). This area has been found with some homicide cases during the past 10 yr (K. M., personal communication).

In this study, nine experiments were conducted, triplicate in each season; hot season during March–May 2013, rainy season during June–October 2013, and dry season during November 2013–February 2014. To protect the decomposition from scavengers such as rat and dog, metal mesh fence with 1.70 m of height and mesh 3 by 3 cm, were set in a 6- by 8-m^2 area (Fig. 1B–D). Temperature (°C), relative humidity (%), and light intensity (lux) were recorded every 20 min by using KIMO data logger (KH-210 AO, United Kingdom), and rainfall (mm^3) was recorded hourly by using home-made rain gauge (∅ 20 cm by 15 cm).

Carrion Model

Male broiler carcasses weighing 1.30–1.70 kg were used as models in this decomposition study. Three trials were conducted in each season. Each trial consisted of three carcasses. All broilers were bought from a nearby local poultry slaughter. Boilers were killed (0530 hours) with a small incision in the jugular vein and feathers removed. At 0600 hours, the featherless carcasses were placed laterally on ground (Fig. 2A) under the fly collection trap (Fig. 2B). Each carcass was placed separately at least 2 m apart from each other. After 72 h (3-d period), mesh of the trap was placed down close to the ground (Fig. 2C).

Visiting Arthropod Collection

Collection of visiting arthropod to broiler carcasses was done hourly for the first 72 h (3-d period) of decomposition by using trap located over the carcasses. The trap was modified from prototype trap (30 by 30 by 50 cm) of Ngoen-Klan et al. (2011) to cover an area of 50 by 50 cm covering the carcass. As for the removable mesh (430 holes/cm^2), it was changed hourly to collect visiting arthropod in an hour interval from sunrise until sunset. Arthropods were trapped for 14 h (0600–2000 hours) during hot and rainy season, but only 12 h (0600–1800 hours) during dry season owing to the short daylight period.

The mesh containing collected arthropods was replaced hourly. The mesh containing collected arthropods was placed into an ethyl acetate bottle for 20 min to anesthetize all flies. Insect specimens of each hour were kept separately in a collection box. If immature stages (eggs and larvae) were present, they were photographed and then transferred to a rearing box containing pork liver.

All collection boxes and rearing boxes were transported to the laboratory. Collection boxes containing adults were placed into the freezer (0 °C) until identification. Immatures were reared in the rearing box with pork liver until developing to adult for species identification.

All specimens were identified using taxonomic key of Triplehorn and Johnson (2005). As for Calliphoridae, Muscidae, and Sarcophagidae, flies were identified to species by using the specific key of Kurahashi and Bunchu (2011) for Calliphoridae; Tumrasvin and Shinonaga (1977, 1978, 1982) and Moophayak et al. (2011) for Muscidae; and Kurahashi and Chaiwong (2013) for male Sarcophagidae. All identified specimens were pinned and kept in the laboratory of Mahidol University, Nakhonsawan Campus.

Statistical Analysis

Statistical analysis was performed by using SPSS 17.0 for Windows. *F*-test was used to determine the difference among environmental factors of hot, rainy, and dry seasons (P-value < 0.05). The correlation of environmental factors with fly population and oviposition and larviposition were determined by using Pearson correlation test (P-value < 0.05).

Results

Environmental Factors Varied Among Seasons

Nine experiments of carrion fly colonization to decomposition were conducted in three replicates of three seasons in Thailand. As shown in Table 1, environmental factors varied across seasons. Ambient air temperature and light intensity presented a significant higher level during hot and rainy season to dry season ($F = 34.136$, 16.066; P-value < 0.01, 0.01, respectively). Some rainfall was found only during rainy season, but relative humidity showed no significant differences to other seasons ($F = 1.728$; P-value $= 0.18$).

Diurnally, sunlight intensity was recorded for 14 h (0600–2000 hours) during hot and rainy seasons and $\leq$13 h (0600–1900 hours) during dry season (Fig. 3). Temperature was greater during daylight hours and peaked during the afternoon. Light intensity showed positive correlation with temperature, particularly during diurnal period (Pearson correlation test, P-value $= 0.969$), but negative correlation was found with relative humidity after 1-h sunshine (Pearson correlation test, P-value $= 0.979$). Rain during the evening and the nocturnal period of the first day was recorded (Fig. 4). Raining resulted in increased relative humidity, thereafter, but no significant correlation was found (Pearson correlation test, P-value $= 0.115$).

Visiting Pattern of Carrion Arthropods to Carcasses

From 27 broiler carcasses, a total of 21,536 arthropods were collected by using trap located over the carcasses. Flies ranked the highest, whereas number of carrion-feeder beetles (Coleoptera), predacious ants and wasps (Hymenoptera), predacious spiders (Arachnida), and accidental-visiting moths (Lepidoptera) were

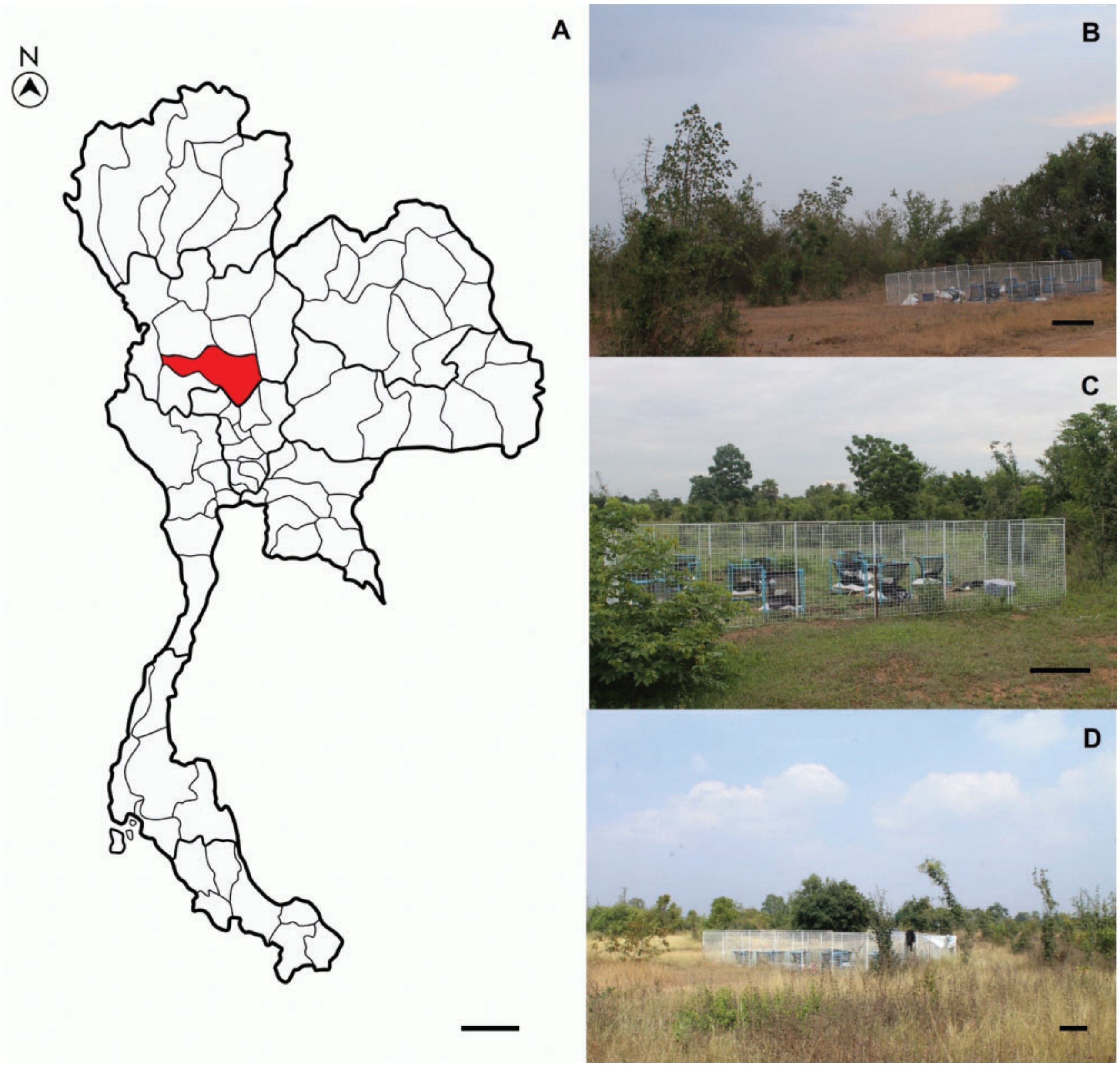

Fig. 1. Location of study site. (**A**) Map of Thailand indicating Nakhonsawan province (15.58189° N; 100.14640° E; bar = 50 km). (**B**) Experimental area in Mahidol University, Nakhonsawan Campus, during hot season (March–May 2013; bar = 10 m). (**C**) Experimental area during rainy season (June–October 2013; bar = 10 m). (**D**) Experimental area during dry season (November 2013–February 2014; bar = 10 m).

found <5% (Table 2). Carrion flies belonging to Calliphoridae (72.92%), Muscidae (18.08%), and Sarcophagidae (2.42%) were the dominant taxa. *Chrysomya megacephala* and *C. rufifacies* were found in every season with the highest number (36.18% and 35.36%, respectively). However, nine other families of Diptera were collected as well. These families, which constituted <6% of specimens collected, included Culicidae, Drosophilidae, Fanniidae, Lonchaeidae, Micropezidae, Platystomatidae, Tachinidae, Tephritidae, and Ulidiidae.

As shown in Table 3, number of carrion flies attracted to carcasses was found largely during rainy season (62.04%), followed by hot (20.02%) and dry seasons (17.93%). Number of carrion flies increased significantly from the first day (1–24 h) to the second day (25–48 h; $F = 12.039$, P-value < 0.01), which was similar to what occurred during the third day (49–72 h; P-value $= 0.68$). Except for number of flies during hot season, no significant difference was found between the second and the third day of decomposition.

Number of carrion flies to visit the broiler carcasses varied by season (Fig. 5). They mostly came to carcasses at the beginning of the experiment (0 h) especially during hot and rainy seasons, but they arrived 1–2 h later during the dry season. *Chrysomya megacephala* and *C. rufifacies* were the first species to visit the carcasses during all seasons.

During the diurnal period of hot and rainy seasons, peak fly visit occurred during late morning (0900–1100 hours) and afternoon (1500–1700 hours). In contrast, the visiting pattern of insects was different during dry season, with a gradual increase throughout the day. No carrion flies were trapped after sunset during any seasons. Only mosquitoes (Culicidae) and moths (Lepidoptera) were collected during the nocturnal period.

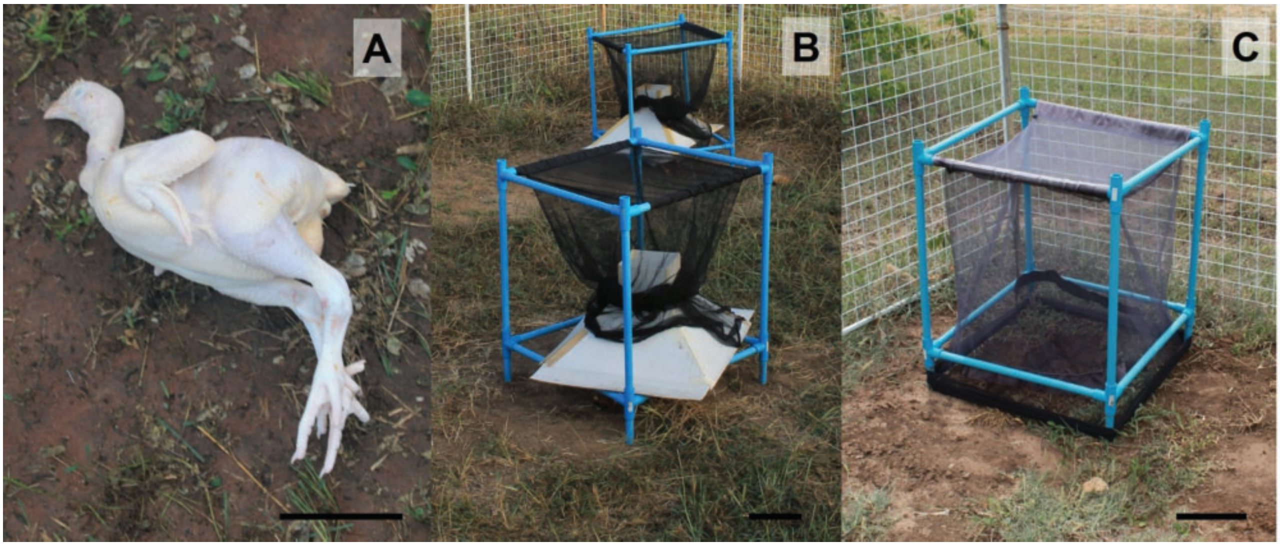

Fig. 2. Broiler carcass and fly trap. (**A**) Placement of broiler carcass (bar = 10 cm). (**B**) Fly collection trap (50 by 50 by 50 cm) placed over broiler carcass during first 3 d of decomposition (bar = 20 cm). (**C**) Fly collection trap with mesh closed to the ground during the 4th to 15th day of experiment (bar = 20 cm).

Table 1. Mean (range) of temperature (°C), relative humidity (%), light intensity (lux), and rainfall (mm^3) at Mahidol University, Nakhonsawan Campus, during hot (March–May 2013), rainy (June–October 2013), and dry seasons (November 2013–February 2014)

Season	Environmental factors			
	Temperature(°C)	Relative humidity (%)	Light intensity (×10^2 lux)	Rainfall(mm^3)
Hot	35.06^a	53.08^a	27.20^a	0.00^b
	(25.81–49.70)	(19.45–86.76)	(0–98.94)	(0.00–0.00)
Rainy	33.55^a	59.35^a	27.11^a	0.14^a
	(25.04–46.40)	(28.40–86.93)	(0–79.18)	(0–1.4)
Dry	25.86^b	56.73^a	15.01^b	0.00^b
	(18.77–34.14)	(27.33–83.47)	(0–42.10)	(0.00–0.00)

Different letter in the same column meaning significant differences (*F*-test, *P*-value < 0.05).

Oviposition and Larviposition of Carrion Flies

There were 36 oviposition and larviposition events by three carrion fly species recorded during the experiments. Colonization by single fly species during decomposition was found the greatest by *C. rufifacies* (40.74%) followed by *C. megacephala* (29.63%). Mixed colonization of fly species was found by *C. rufifacies* and *C. megacephala* (22.22%), as well as *C. megacephala* and *P. dux* (7.41%). No more than two fly species were colonized together in the carcasses.

As shown in Fig. 6, *C. rufifacies* was the first species which laid their eggs on the carcasses at 1500 hours (9 h). *Chrysomya rufifacies* and *C. megacephala* oviposited on the remains consistently during similar times (1600–1700 hours) in all seasons. Of the 18 oviposition events by *C. rufifacies*, 66.67% were found during the afternoon (1600–1700 hours) of the first day. Likewise, oviposition time of *C. megacephala* mostly (80%) occurred during the afternoon (1600–1700 hours). Few numbers of both fly species (30.55%) oviposited later in the second day, especially during dry season (22.22%; Figs. 7–9).

Larviposition by *P. dux* was found later (23–27 h) than oviposition of calliphorid flies and occurred in the second day of rainy and dry seasons. One hundred percent of broiler carcasses were colonized by *C. rufifacies* and *C. megacephala* on day one during hot season, but 11.11% and 77.78% of broiler carcasses were colonized in the second day during rainy and dry season, respectively. At least 22% of carcasses were food sources for development of two species: 1) between *C. megacephala* and *C. rufifacies*, and 2) *C. megacephala* and *P. dux*. Females of *C. megacephala* mostly laid their eggs after coincidence species. Moreover, only three batches of *C. megacephala* and *C. rufifacies* were found in the morning (0800–1000 hours).

Discussion

We determined TOC differs from TOP and TOD of broiler carcasses across seasons in Nakhonsawan Province, Thailand. Variation between TOC and TOP of *C. rufifacies* and *C. megacephala* ranged from 9 to 11 h during hot season, while 10 to 36 h during rainy and dry seasons. Variation between TOC and TOP of *P. dux* ranged from 35 to 47 h during rainy and dry seasons. This information is significant for the field of forensic entomology, as an appreciation for such variation could be applicable for determining a more accurate PMI during forensic investigations.

Furthermore, although experiments on insect succession on animal carcasses have been examined in Thailand, only two studies were reported from Phitsanulok and Nan Provinces in the north (Vitta et al. 2007, Sukchit et al. 2015). Because geographical area plays an important role in insect succession, the importance of the present study is increased, as it was the first study on insect succession in Nakhonsawan, upper-central Thailand, as well as the first

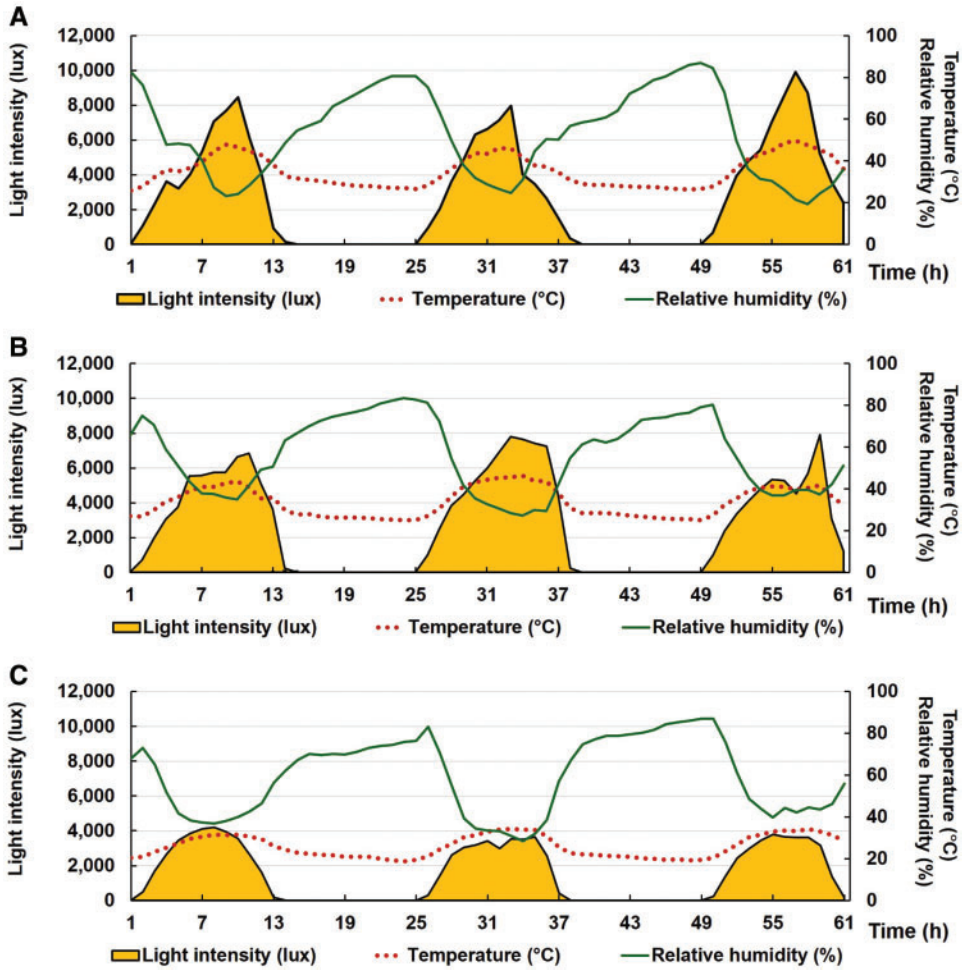

Fig. 3. Mean hourly occurrences of environmental factors: temperature (°C), relative humidity (%), and light intensity (lux) at the study site in Mahidol University, Nakhonsawan Campus, during (**A**) hot season (March–May 2013), (**B**) rainy season (June–October 2013), and (**C**) dry season (November 2013–February 2014).

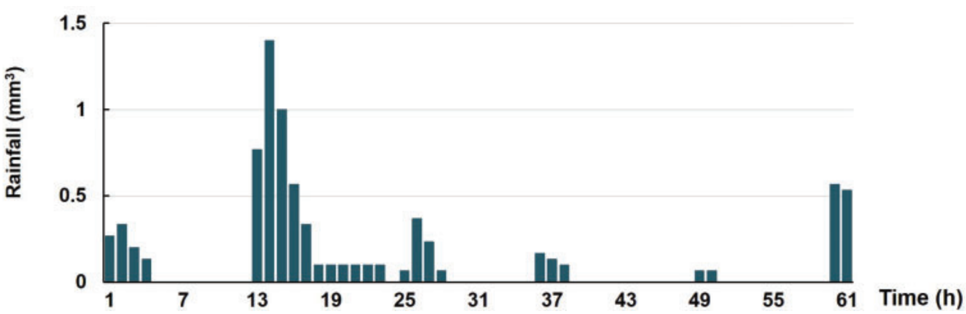

Fig. 4. Mean hourly occurrences of rainfall (mm^3) at the study site in Mahidol University, Nakhonsawan Campus, during the rainy season (June–October 2013).

study to determine the timing of fly colonization of vertebrate carrion.

Abundance and succession of arthropods, especially carrion flies, in association with broiler carrion decomposition were significantly affected by environmental conditions. This current study revealed that among the 21,536 arthropods trapped over the carcasses, carrion fly species belonging to three families (i.e., Calliphoridae, Muscidae, and Sarcophagidae) were dominant. In fact, two forensic important species, *C. rufifacies* and *C. megacephala*, ranked the first and the second most collected in all seasons, respectively. These results were similar to experiments previously conducted in Thailand with pig carcasses (Vitta et al. 2007, Sukchit et al. 2015) and pork or beef viscera (Ngoen-Klan et al. 2011, Bunchu et al. 2012, Klong-Klaew et al. 2014). Furthermore, data are similar to what has been associated with actual forensic cases in Malaysia (Lee et al. 2004), indicating broader implications of our study.

Greater numbers were collected during the rainy rather than hot season. These differences could be owing to the variation in temperature, light intensity, relative humidity, and rainfall experienced across seasons (Campobasso et al. 2001). A large number of flies were collected during high temperature (30–40 °C), but a few number of flies were collected when high level of relative humidity

Table 2. Amount numbers (%) of arthropods visited 3-d decomposition at Mahidol University, Nakhonsawan Campus, during March 2013–February 2014

Class	Order	Family	Genus species	Amount (%)
Hexapoda	Diptera	Calliphoridae	*Chrysomya nigripes*	156 (0.72)
			Chrysomya megacephala	7,792 (36.18)
			Chrysomya rufifacies	7,615 (35.36)
			Hemipyrellia ligurriens	34 (0.16)
			Hemipyrellia pulchra	34 (0.16)
			Phumosia spp.	2 (0.01)
			Rhyncomya flavibasis	5 (0.02)
			Stomorhina discolor	63 (0.29)
			Stomorhina siamensis	2 (0.01)
		Muscidae	*Atherigona orientalis*	20 (0.09)
			Atherigona spp.	1,039 (4.82)
			Hydrotaea chalcogaster	48 (0.22)
			Hydrotaea spinigera	171 (0.79)
			Musca domestica	835 (3.88)
			Musca pattoni	38 (0.18)
			Musca sorbens	1,219 (5.66)
			Musca ventrosa	511 (2.37)
			Musca spp.	13 (0.06)
		Sarcophagidae	*Boetcherisca peregrina*	12 (0.06)
			Liopygia ruficornis	71 (0.33)
			Parasarcophaga dux	91 (0.42)
			Parasarcophaga hirtipes	25 (0.12)
			Parasarcophaga misera	64 (0.30)
			Sarcophaga spp.	258 (1.20)
		Culicidae		154 (0.72)
		Drosophilidae		2 (0.01)
		Fanniidae		5 (0.02)
		Lonchaeidae		70 (0.33)
		Micropezidae		1 (0.00)
		Platystomatidae		5 (0.02)
		Tachinidae		21 (0.10)
		Tephritidae		2 (0.01)
		Ulidiidae		944 (4.38)
	Coleoptera			15 (0.07)
	Hymenoptera			48 (0.22)
	Lepidoptera			110 (0.51)
Arachnida				41 (0.19)
Total				**21,536 (100)**

Table 3. Mean numbers (range) of carrion flies (including Calliphoridae, Muscidae, and Sarcophagidae) collected from Mahidol University, Nakhonsawan Campus, Thailand

Decomposition	Season[a]			Amount
	Hot	Rainy	Dry	
The first day	19.88[b+] (0–74)	47.08[b++] (0–193)	23.79[b+] (0–86)	30.25[b] (0–193)
The second day	73.17[a+] (0–262)	191.38[a++] (0–477)	50.79[ab+] (0–266)	105.11[a] (0–477)
The third day	74.79[a+] (0–222)	281.63[a++] (0–666)	75.75[a+] (0–239)	93.14[a] (0–666)
Total number (%)	**4,028 (20.02)**	**12,482 (62.04)**	**3,608 (17.93)**	**20,118 (100)**

Different letter ([a, b]) in the same column meaning significant differences (*F*-test, *P*-value < 0.05).

Different symbol ([+, ++]) in the same row meaning significant differences (*F*-test, *P*-value < 0.05).

[a] Hot (March–May 2013, rainy (June–October 2013), and dry seasons (November 2013–February 2014).

(40–60%) was present . The positive correlation between number of flies and temperature, and the negative correlation with relative humidity of this study were similar to the results from previous field experiments studied on *C. megacephala*, *M. domestica*, and *C. rufifacies* in many land-use types (e.g., disturbed mixed deciduous forest, mixed deciduous forest, mixed orchard, lowland village, city town, and paddy field) of Chiang Mai by Ngoen-Klan et al. (2011) and Klong-Klaew et al. (2014). Yanmanee et al. (2016) determined immature development of *C. rufifacies* was completed at 15–36 °C, but no adult emergence occurred when rearing at a constant 39 °C. No correlation with light intensity was revealed from *C. megacephala* or *M. domestica* activity (Ngoen-Klan et al. 2011); in contrast, a positive correlation was determined for *C. rufifacies* (Klong-Klaew et al. 2014). Ahmad et al. (2011) conducted a decomposition experiment in Malaysia and determined flies moved away from carcasses when light intensity was too low as experienced during twilight, which was similar to results from the current study. By using a wind tunnel, minimal light intensity yielded lower behavioral responses of *C. megacephala* than those having light (Moophayak et al. 2013). More sufficient light intensity can activate flies to make

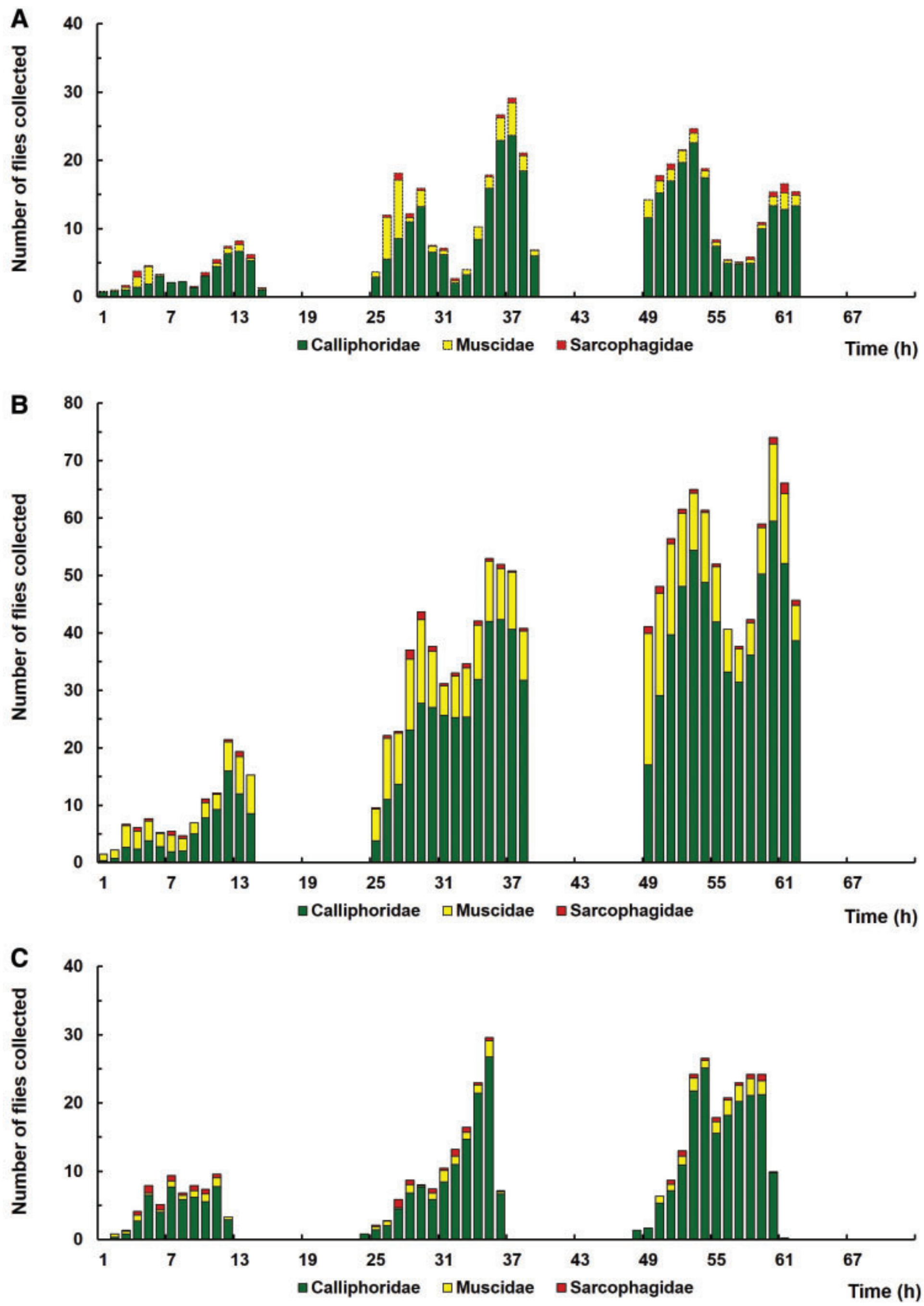

Fig. 5. Hourly visiting pattern of three carrion flies: Calliphoridae, Muscidae, and Sarcophagidae to 3-d decomposition at the study site in Mahidol University, Nakhonsawan Campus, during (**A**) hot season (March–May 2013), (**B**) rainy season (June–October 2013), and (**C**) dry season (November 2013–February 2014).

the greater visibility for landing (Archer and Elgar 2003, Burkett and Butler 2005). Thus, shorter hour of sunrise, ~2 h (Agrometerological data, Takfa Agromet Station, Nakhonsawan Thailand, 2013), reduced the light intensity during dry season in Thailand and yielded minimal activity of flies.

As for rainfall, number of flies collected tended to decrease during such events, which is similar to a previous study by Mahon et al. (2004). Sukchit et al. (2015) also determined the numbers of fly collected were less (2,430 insects) at Nan province, Thailand, during wet season than hot (5,061 insects) and dry season (4,233 insects).

According to the current study, *C. rufifacies* or *C. megacephala* was the first insect to visit fresh broiler carcasses within an hour after placement in the field at 0600 hours. Vitta et al. (2007) and Sukchit et al. (2015) also recorded similar results when using pig carcasses in decomposition studies in Thailand. It should be noted no nocturnal oviposition or larviposition was found in this study. However, it should be noted artificial light could activate flies (Zurawski et al. 2009), which could change the outcomes observed in this study.

Based on previous case reports in Thailand, Sribanditmongkol et al. (2014) determined *C. rufifacies* coincidence with other blow

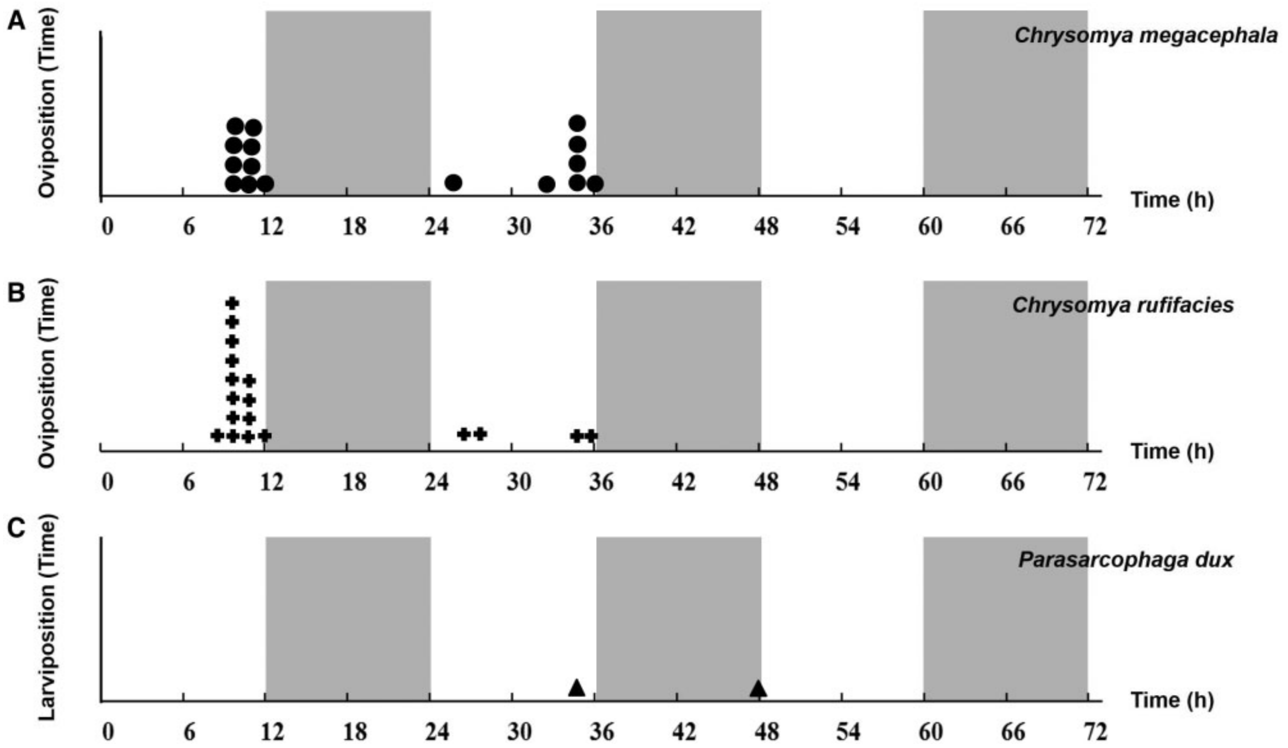

Fig. 6. Hourly oviposition and larviposition at study site in Mahidol University, Nakhonsawan Campus, during initial decomposition during March 2013–February 2014 of *C. megacephala* (•), *C. rufifacies* (+), and *P. dux* (▲).

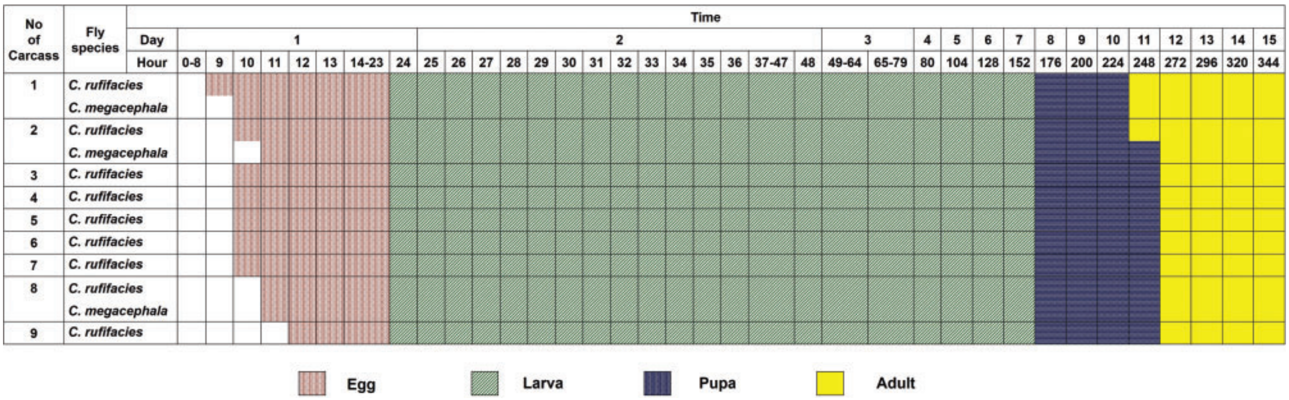

Fig. 7. Carrion fly development (*C. megacephala* and *C. rufifacies*) in broiler carcasses at the study site in Mahidol University, Nakhonsawan Campus, during the hot season (March–May 2013).

flies, such as *C. megacephala*. Furthermore, in this study, *C. rufifacies* oviposited more often than *C. megacephala* or *P. dux* (larviposit). Goff (2000) determined *C. rufifacies* will lay eggs after *C. megacephala*. The current study recorded similar results. This behavior could result in greater success for *C. rufifacies*, as their larvae are predaceous on *C. megacephala* larvae (Yang and Shiao 2012).

In this study, more than a half of forensic important species (i.e., *C. megacephala*, *C. nigripes*, *C. rufifacies*, *H. ligurriens*, *H. spinigera*, and *L. ruficornis*) and forensic-related species (i.e., *B. peregrina*, *M. domestica*, *M. sorbens*, and *P. dux*) visited the broiler carcasses. On the other hand, some species were not included. There may be two possible explanations. First, broiler carcass is not a suitable model to examine insect taxa (Weidner et al. 2015). Domestic pigs (*Sus scrofa domesticus*) have been used as surrogates for human cadavers during forensic experiments owing to similarities in physiology (Tomberlin et al. 2011). Small size of carcass might not produce strong potential of olfactory cue to attract all forensic flies (Weidner et al. 2015), then yielding the result of some carrion species of forensic importance were missing. Second, the geographical area of the study was a disturbed forested area which was located at lowland (82.88 m above sea level) and nearby (1–2 km far) residential areas; thus, synanthropic carrion species were found, as previous studies in Chiang Mai (Ngoen-Klan et al. 2011, Klong-Klaew et al. 2014), Phitsanulok (Bunchu et al. 2012), and Ubonratchathni (Chaiwong et al. 2012). However, two highly synanthropic flies of *L. cuprina* and *S. nudiseta* were not included. It might be that they were distributed only in and around urbanized areas (Bunchu et al. 2012, Chaiwong et al. 2012). On the other hand, the other species of *C. bezziana*, *C. chani*, *C. pinguis*, *C. thanomthani*, *C. villeneuvi*, and *H. infumata* are likely to prevail in the forested areas, in

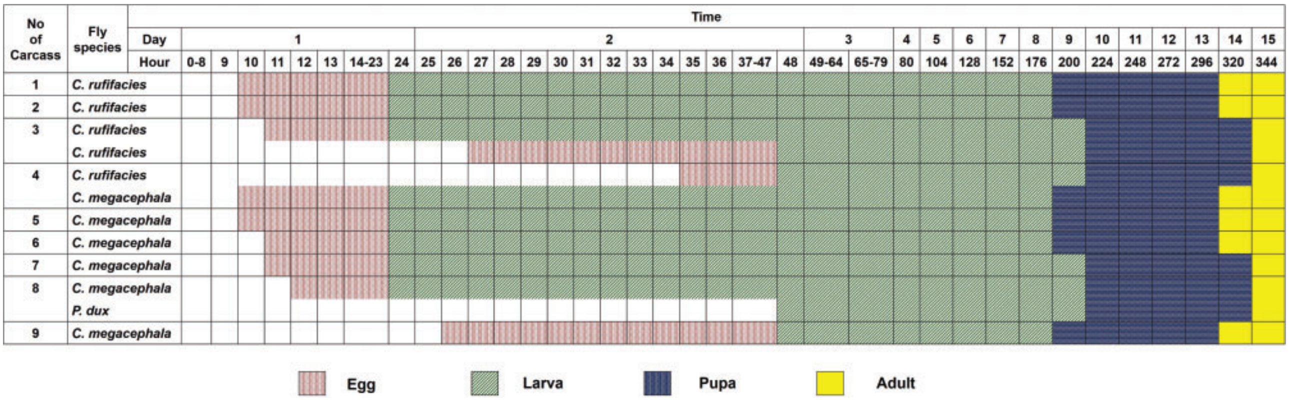

Fig. 8. Carrion fly development (*C. megacephala*, *C. rufifacies*, and *P. dux*) in broiler carcasses at the study site in Mahidol University, Nakhonsawan Campus, during the rainy season (June–October 2013).

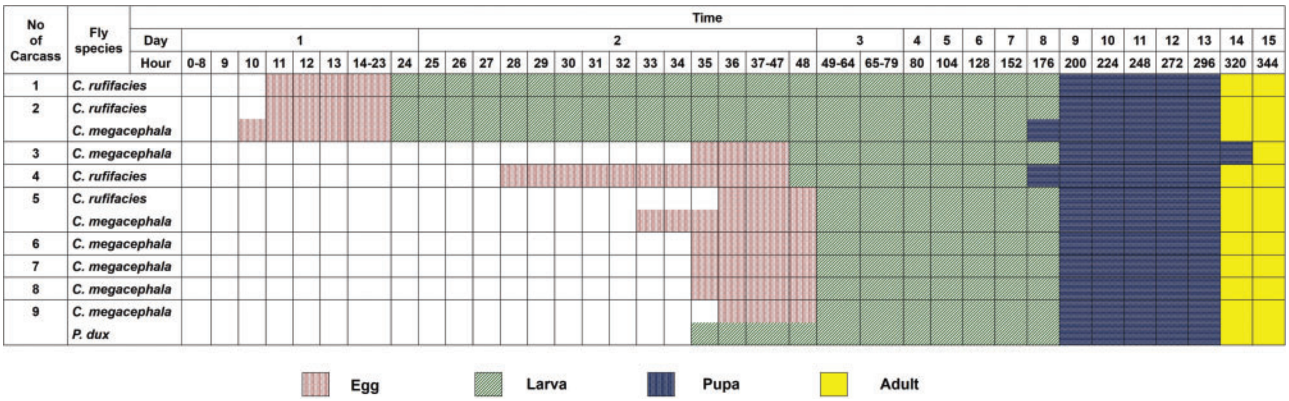

Fig. 9. Carrion fly development (*C. megacephala*, *C. rufifacies*, and *P. dux*) in broiler carcasses at the study site in Mahidol University, Nakhonsawan Campus, during the dry season (November 2013–February 2014).

particular with high altitude (>450 m above sea level; Bunchu et al. 2012, Moophayak et al. 2014).

Even though broiler carcass is not a suitable model, but in this situation, it has an advantage of monitoring the minimal period between TOD and TOP constantly at 0.5 h to focus on TOC, and also multiple replicates for each trial were possible. The model ranked the third most used in forensic examination (Tomberlin et al. 2012) and suitable for development of carrion fly larvae (Swiger et al. 2014). In addition, species diversity of insect during the initial stage of decomposition between chicken and pig carcasses was not significantly different (Arnaldos et al. 2015).

In conclusion, the results of this study indicate that TOC is different from TOP and TOD. Therefore, such variation needs to be addressed when attempting to make PMI estimates with entomological evidence. Future research by using pig carcass as model would need to continue to explore these factors to enhance the accuracy of forensic entomology in future forensic cases.

Acknowledgments

This work received financial support from the Mahidol University Fund for newly teaching staff. Our sincere thanks to Dr. Hiromu Kurahashi (International Department of Dipterology, Japan) and Dr. Tarinee Chaiwong (Ubonratchathani University, Thailand) for identification of flies. Thankful acknowledgement to Dr. Supaporn Kamruangrit (Mahidol University, Nakhonsawan Campus) and all staffs of Mahidol University, Nakhonsawan Campus, for their kind support during the study.

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