Daily and Seasonal Prevalence of the Blow Fly Chrysomya Rufifacies (Diptera: Calliphoridae) as Revealed by Semiautomatic Trap Collections in Suburban Chiang Mai Province, Northern Thailand

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Daily and seasonal prevalence of the blow fly *Chrysomya rufifacies* (Diptera: Calliphoridae) as revealed by semi-automatic trap collections in suburban Chiang Mai Province, northern Thailand

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Abstract

Effective control of *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae), a blow fly species of medical and forensic importance, requires information on seasonal prevalence and bionomics. Therefore, daily and seasonal activity patterns of *C. rufifacies* were studied in 3 locations representing different microhabitats (palm plantation, forested area, longan orchard) in a suburban area of Chiang Mai Province, northern Thailand. Investigations were conducted hourly for 24 h using a semi-automatic trap baited with 1-d-old beef offal (300 g). Collections were carried out twice per mo from Jul 2013 to Jun 2014. A total of 55,966 adult *C. rufifacies* were collected, with 52.4% of individuals trapped in the forested area. *Chrysomya rufifacie*s was present in collections throughout the yr with peak abundance in summer. This species was active during the d with peak activity in late afternoon (3:00 to 6:00 PM). Fly abundance in traps was positively correlated with temperature (r = 0.391; $P < 0.001$) but negatively correlated with relative humidity (r = −0.388; $P < 0.001$). Female flies were more abundant in collections (0.26 male per female sex ratio), with 80% of individuals being non-gravid. The baseline information provided by our study suggests that *C. rufifacies* is well-adapted to variable climatic conditions present in northern Thailand, specifically suburban Chiang Mai Province.

Key Words: daily activity; seasonal activity; hairy maggot blow fly; fly abundance

Resumen

El control efectivo de *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae), una especie de mosca califórida de importancia médica y forense, requiere información sobre la prevalencia estacional y bionómica. Por lo tanto, se estudiaron los patrones de actividad diaria y estacional de *C. rufifacies* en tres lugares que representan diferentes microhábitats (plantaciones de palma, área boscosa, huerto de ojo de dragón) en un área suburbana de la provincia de Chiang Mai, al norte de Tailandia. Se realizaron las investigaciones cada hora durante 24 h utilizando una trampa semiautomática cebada con despojos de carne de 1 día de edad (300 g). Se hicieron las recolecciones dos veces al mes entre jul del 2013 y jun del 2014. Se recolectó un total de 55,966 adultos de *C. rufifacies*, con el 52,4% de los individuos atrapados en el área boscosa. *Chrysomya rufifacies* estuvo presente en colecciones a lo largo del año con una abundancia máxima en el verano. Esta especie estuvo activa durante el día con una actividad máxima al final de la tarde (3:00 to 6:00 PM). La abundancia de moscas en las trampas se correlacionó positivamente con la temperatura (r = 0.391, $P < 0.001$) pero se correlacionó negativamente con la humedad relativa (r = -0.388, $P < 0.001$). Las moscas hembra fueron más abundantes en las colecciones (proporción sexual machos/hembras de 0,26) con un 80% de individuos no grávidos. La información de referencia proporcionada por nuestro estudio sugiere que *C. rufifacies* está bien adaptada a las condiciones climáticas variables presentes en el norte de Tailandia, específicamente en la provincia suburbana de Chiang Mai.

Palabras Clave: actividad diaria; actividad estacional; mosca peluda; abundancia de moscas

The hairy maggot blow fly, *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae), is a medically and forensically important species worldwide. This fly is well-adapted to variable environments, ranging from urban regions to the high mountainous zone (Moophayak et al. 2014). In urban areas of Malaysia, adults of this species can be mechanical carriers of various pathogens such as bacteria, viruses, protozoan cysts, and helminth eggs (e.g., *Ascaris lumbricoides* [Ascarididae], *Trichuris trichiura* [Trichuridae]) (Sulaiman et al. 1988). Also, larvae of *C. rufifacies* have been reported as myiasis-producing agents in humans and animals. In Thailand, *C. rufifacies* human myiasis cases sometimes

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coincide with other blow fly species, such as *Chrysomya megacephala* (F.) (Diptera: Calliphoridae) (Sukontason et al. 2005), or *Lucilia eximia* (Wiedemann) (Diptera: Calliphoridae) in the US (Sanford et al. 2014). On sheep in Australia, where *C. rufifacies* is native, larvae are regarded as a secondary myiasis producer because this species normally does not strike sheep until the primary maggot invaders are already feeding (Baumgartner 1993). *Chrysomya rufifacies* can be a forensically important species, because larvae are capable of primarily colonizing human remains (Sukontason et al. 2007; Sribanditmongkol et al. 2014; Syamsa et al. 2015). The first instars of *C. rufifacies* are entirely necrophagous, but under crowded or starving conditions the second and third instars may prey on larvae of other resident carnivorous flies found in myiasis situations. Therefore, *C. rufifacies* could possibly be considered to be a biological control agent by reducing nuisance and disease-carrying blow fly populations (Baumgartner 1993).

In Thailand, *C. megacephala* and *C. rufifacies* coexist in various ecological environments, including urban, rural, and forested areas (Ngoen-klan et al. 2011; Klong-klaew et al. 2014). Both flies are regarded as ecologically similar species (i.e., species that use the same resource) (Sukontason et al. 2003; Ngoen-klan et al. 2011; Klong-klaew et al. 2014). Larvae of *C. rufifacies* were reported to attack *C. megacephala* in a forensic entomology field study and in the laboratory (Wells & Greenberg 1994; Wells & Kurahashi 1997). Nonetheless, there has been no evidence of larval competition between these 2 ecologically similar species in forensic investigations in Thailand.

Many studies have been carried out to determine the ecology of blow flies by focusing on their relative abundance in different seasons and habitats. Such knowledge provides an important basis for applied research (e.g., control strategy) and forensic investigations. Nevertheless, this information is usually limited to specific study areas. Generally, the ecology of *C. rufifacies* is poorly understood, particularly in relation to ecological factors that affect population dynamics within microhabitats (Zabala et al. 2014).

Precise information on the annual activity of forensically important flies is necessary for estimation of minimum post-mortem interval (PMI_{min}), especially in determining the time of death. Abiotic factors such as temperature, relative humidity, light intensity, and rainfall have been reported to impact the distribution of *C. rufifacies* (Vogt 1988; Klong-klaew et al. 2014). Furthermore, a complex interaction between the timing of the daily light-dark cycle and temperature is the principal factor influencing insect activity (Archer & Elgar 2003). Although the seasonal distribution of *C. rufifacies* has been studied in Australia (Norris 1966; Mcleod & Anderson 1992) and Thailand (Klong-klaew et al. 2014), the distribution and abundance of this species, particularly the diurnal cycle and seasonal variability, have not yet been studied in northern Thailand. In order to gain a better understanding of the population dynamics of this fly, we collected daily and seasonal activity patterns of adult *C. rufifacies* in relation to ambient climatic factors (temperature, relative humidity) in Chiang Mai Province, northern Thailand. In addition, we also obtained information on the ecological relationship between *C. rufifacies* and *C. megacephala* populations under natural conditions.

Materials and Methods

STUDY SITE

This study was conducted at Mae Hia Agricultural Research, Demonstrative, and Training Center, Chiang Mai Province, northern Thailand. Sampling occurred in (i) a forested area (18.766966°N, 98.935638°E, elevation 344 masl), located in the foothills of a mixed deciduous forest that contained teak (*Tectona grandis* L.f.) (Lamiaceae) and various bushes (e.g., *Mimosa pudica* L.) (Fabiaceae); (ii) a palm plantation (18.757733°N, 98.930143°E, elevation 330 masl), consisting mainly of Tenera palm trees (*Elaeis guineensis* Jacq.) (Arecaceae); and (iii) a longan, *Dimocarpus longan* Lour. (Sapindaceae), orchard (18.765738°N, 98.927813°E, elevation 347 masl).

FLY COLLECTION

Five semi-automatic traps, previously described by Klong-klaew et al. (2017), were used to monitor adult *C. rufifacies* abundance. Briefly, the trap consisted of a rectangular metal case (40 × 40 × 60 cm) fitted with a mesh net (36 × 36 × 85 cm) using an elastic band that fits over the trap entrance. A square funnel fly entrance module, made of transparent plastic board, was connected to a modified CD player with a sliding tray to facilitate rotating independent collections controlled by a timer. Collections were conducted during a 24 h period at the intervals shown in Table 1. Traps were baited with 300 g of beef offal previously held for 24 h at ambient temperature. The offal was obtained from the same butcher shop and prepared in the same manner throughout the experiment period. Bait age coincided with collection intervals to insure 24-h-old offal (Table 1) at each time period. To prevent contamination, each bait was placed in a separate container at ambient temperature. Offal bait has been shown previously to be effective in attracting medically important blow flies in the field (Ngoen-klan et al. 2011; Klong-klaew et al. 2014). All fly collections remained in the field until the trap had completed its 24 h rotation. The contents of each trap were manually emptied by removing the fly net from the external metal case and installing a new net for the next 24 h collection. To exclude scavengers and prevent rain damage to collections, traps were placed inside wire cages where the top portion was covered with transparent plastic sheets. Also, to prevent ants and other crawling insects from entering the traps, the leg of each trap was placed in a transparent plastic tray filled with water. Five traps were set out in each of the 3 study sites and collections were conducted twice per mo from Jul 2013 to Jun 2014 for a total of 360 samples obtained for the entire study. During each experiment, hourly temperature (°C) and relative humidity (RH, %) were recorded using Ebro EBI 20-TH1 data loggers (Ebro Electronic GmbH & Co. KG, Ingolstadt, Germany). Mean monthly minimum and maximum temperatures and rainfall information were obtained from the Chiang Mai weather station, whereas daily sunrise and sunset data was obtained from the Thai Meteorological Department (Mueang Chiang Mai district, central Chiang Mai Province).

Fly collections were transferred to the laboratory at the Department of Parasitology, Faculty of Medicine, Chiang Mai University, for identification using the taxonomic keys of Kurahashi and Bunchu (2011). Female *C. rufifacies* were dissected to determine ovarian developmental status (gravid vs. non-gravid). We also examined random samples of gravid *C. rufifacies* to determine the number of mature oocytes present by counting the number of eggs in those individuals (Roy & Siddons 1939). Females were dissected under a stereo microscope (Model SZ2-ILST, Olympus Corporation, Tokyo, Japan) at 3× magnification and the status of ovarian development was classified as described

Table 1. Semi-automatic trap operation time and duration of trapping period.

Time period	Trap periodicity	Open	Closed	Duration (h)
Day	Early morning	6:00 AM	9:00 AM	3
	Late morning	9:00 AM	12:00 Noon	3
	Early afternoon	12:00 Noon	3:00 PM	3
	Late afternoon	3:00 PM	6:00 PM	3
Night	Night	6:00 PM	6:00 AM	12

by Chaiwong et al. (2012) for *C. megacephala*. Gravid ovaries generally are covered with thin, fragile ovarian envelopes and have fewer tracheoles. The ovaries filled with mature eggs are elongated.

DATA ANALYSIS

Prior to data analysis, fly numbers were log-transformed [$\log_{10}$ (n + 1)] to fit a normal distribution, but logs were back-transformed into actual numbers for presentation in text and tables. One-way analysis of variance (ANOVA) followed by a post-hoc Bonferroni test (homogeneity of variance: $P > 0.05$) or a Dunnett's T3 test (homogeneity of variance: $P < 0.05$) were performed to compare the mean trap catch in (i) different microhabitats (forested area, palm plantation, and longan orchard), and (ii) different trapping periods.

The mean trap catch among seasons was compared to establish if there was a seasonal trend or habitat preference in each season. To analyze seasonal catch variability, the mean trap catch of the pooled data from 3 study sites was calculated. One-way ANOVA followed by post-hoc tests were employed to compare the mean trap catch of *C. rufifacies* in each season (summer, rainy, and winter).

Bivariate correlation analysis and Pearson correlation coefficient (r) were analyzed to investigate the relationship between trap catch and abiotic factors (temperature and relative humidity) that were recorded locally. Furthermore, bivariate correlation analysis and Spearman's rank correlation coefficient (ρ) were employed to compare the relationship between fly numbers and weather factors (mean temperature and annual rainfall) obtained from the Thai Meteorological Department.

Sex ratio of the collected flies was calculated by using the total number of males divided by the total number of females. Mean egg number in gravid females was compared using 1-way ANOVA followed by Dunnett's T3 post hoc test. Day length was defined as the time from sunrise to sunset. All data were analyzed using SPSS 12.0 Windows (α = 0.05) (SPSS Inc., Chicago, Illinois, USA) and JMP®, Version 11 (SAS Institute Inc., Cary, North Carolina, USA).

Results

A total of 55,966 *C. rufifacies* specimens were collected during Jul 2013 to Jun 2014. The majority of individuals were trapped in the forested area (52.4%) followed by the palm plantation (27.2%) and the longan orchard (20.4%) (Table 2). Mean number of *C. rufifacies* collected was significantly different among seasons, with peak populations trapped in summer (mid-Feb to mid-May) (63.5%), with a sharp decrease in the rainy season (mid-May to mid-Oct) (25.7%) that continued throughout winter (mid-Oct to mid-Feb) (10.8%) (Fig. 1A).

A strong positive relationship was observed between the collection abundance in traps for *C. rufifacies* and *C. megacephala* (r = 0.911; $P < 0.001$) (Fig. 2). Mean *C. rufifacies* abundance in traps was significantly affected only by temperature (ρ = 0.544; $P = 0.006$). No correlation between trap catch and ambient rainfall was found for this species (ρ = −0.236; $P = 0.267$).

During summer, significantly more *C. rufifacies* were captured in traps in the forest compared with the palm plantation ($P = 0.015$) and longan orchard ($P = 0.001$). On the other hand, during the rainy season and winter there was no significant difference in the mean numbers of flies caught among the 3 study sites.

Higher numbers of females *C. rufifacies* (n = 44,001; 78.6%) were captured than males (n = 11,965; 21.4%), resulting in a sex ratio of 0.26 male per female with about 80% of the trapped females being non-gravid (63%). The dissection of ovaries indicated that the mean numbers of eggs from females trapped in the summer and the rainy season were significantly greater compared with those in winter ($P = 0.004$ and $P = 0.003$, respectively) (Table 2).

Based on year-round collections, the greatest trap catch of *C. rufifacies* was obtained from 3:00 PM to 6:00 PM (Fig. 3A). In summer, most flies were trapped during this same time period. Interestingly, the peak catches of *C. rufifacies* occurred from 12:00 Noon to 3:00 PM in the rainy season and winter (Fig. 3A). Few flies were captured during the night period 6:00 PM to 6:00 AM.

Discussion

Although the distribution pattern of *C. rufifacies* has been documented from previous investigations in Australia and Thailand (Norris 1966; Mcleod & Anderson 1992; Klong-klaew et al. 2014), our study is the first to characterize the daily and seasonal activity of adult *C. rufifacies* using a semi-automatic trap in Chiang Mai Province, Thailand. Most of the adult *C. rufifacies* in traps were obtained from the forested area, which may indicate a preference by this species. The forested area may contain a greater variety of plant species that provide shaded and resting areas for adult flies when compared with the other 2 environments. Another potential factor may be the existence of grass-fed cows and other animals (natural dung and carcasses) in the immediate area, making it more attractive to *C. rufifacies* either as food resources or shelter for larvae and adults. A similar occurrence also was observed in forested areas by Bunchu et al. (2012) and Klong-klaew et al. (2014) in Chiang Mai and Phitsanulok Provinces, Thailand. However, a report from Australia by Palmer (1980) indicated that *C. rufifacies* preferred open pasture over forested habitat. The reason for this difference in northern Thailand is unclear.

In the palm plantation, a bimodal fly population curve was observed with a major peak in summer and a minor peak in winter. At this site, the incidence of sunlight is limited by the closed canopy. This may restrict the occurrence of *C. rufifacies* that previously showed a positive relationship with light intensity (Klong-klaew et al. 2014).

In the longan orchard, a bimodal population curve also was observed, with a major peak activity in summer and a minor one in the rainy season when harvesting of longan fruit occurs. High numbers of *C. rufifacies* were captured in Jan 2014, when flowering of the longan trees occurs (Dec 2013–Feb 2014). Trap abundance probably reflects the presence of adult *C. rufifacies* seeking carbohydrates from flower nectar to provide energy for behavioral activities (e.g., flight, copulation) during that time (Norris 1965).

Table 2. Mean (± SEM) adult *Chrysomya rufifacies* trap catch, and mean number of eggs per gravid female in each season, Jul 2013 to Jun 2014.

Season	Number of trap collections	Mean adults[a]	Mean eggs	Total females examined	Min-max eggs per female
Summer	72	482.3 ± 58.9 a	165.3 ± 1.50 a	855	58 – 292
Rainy season	120	114.3 ± 12.7 b	167.2 ± 2.37 a	511	45 – 300
Winter	96	62.8 ± 10.6 c	154.7 ± 2.96 b	303	50 – 288

[a]Means in a column followed by different letters are significantly different (Dunnett's T3 post hoc test; $P < 0.05$).

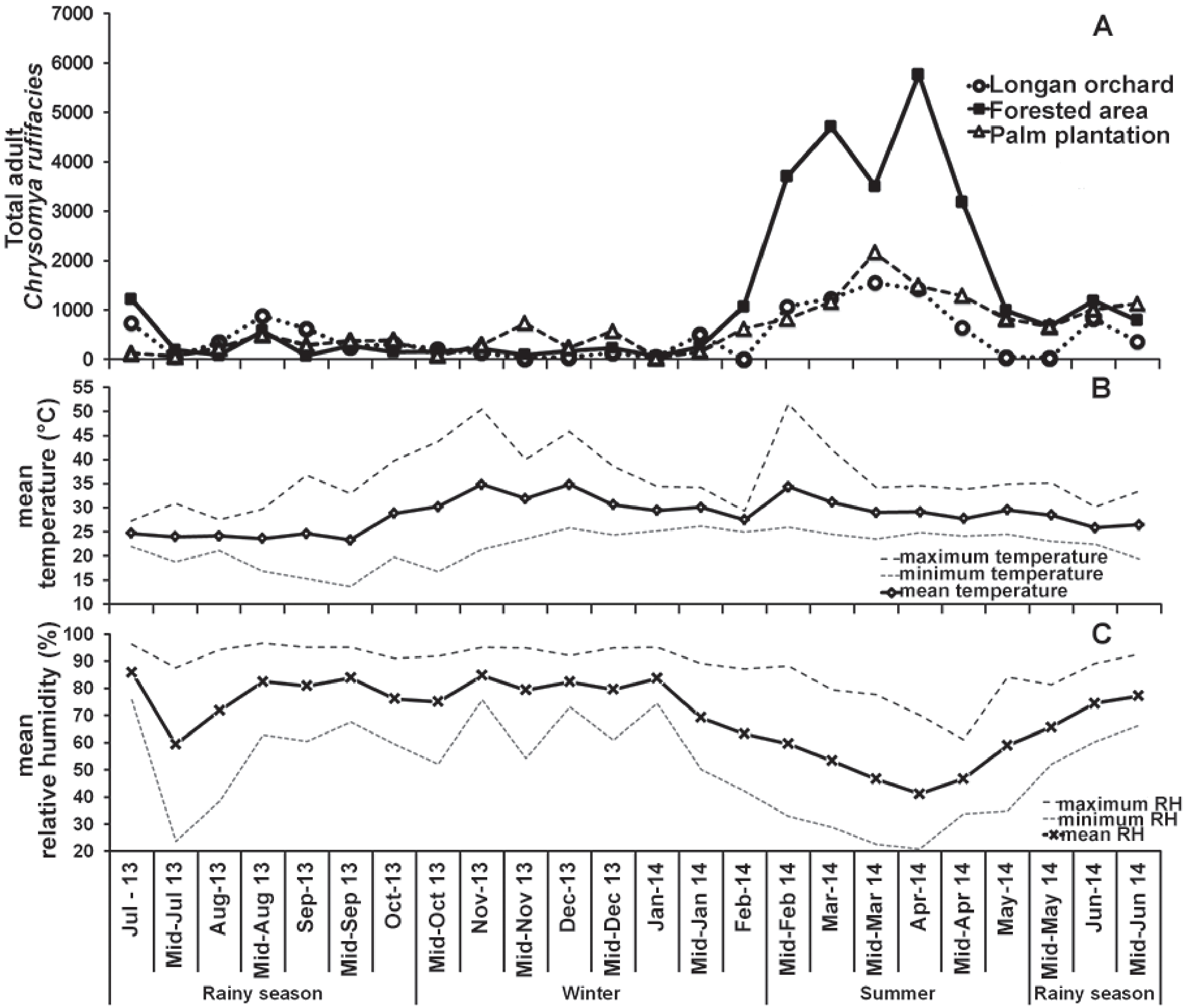

Fig. 1. (A) Seasonal fluctuation of total adult *Chrysomya rufifacies* collected by semi-automatic traps; (B) Mean monthly temperature; and (C) Mean relative humidity recorded at the study sites in Chiang Mai Province, northern Thailand during Jul 2013 to Jun 2014.

We also found that fly collections were greater in the summer compared with the other seasons, and it may be the fact that the 1-d-old beef offal used as a bait in this study emits stronger odors during hot periods, thereby playing an important role in attracting adult flies (Bunchu et al. 2008). Furthermore, use of this bait also favored collection of females, more so than male flies, as observed before with *C. rufifacies* and *C. megacephala* on meat-baited traps (Lertthamnongtham et al. 2003; Ngoen-klan et al. 2011; Klong-klaew et al. 2014).

As mentioned earlier, second and third instar cyclorrhaphan Diptera can be facultative predators of other dipteran larvae. Goodbrod and Goff (1990) and Baumgartner (1993) suggested that larval *C. rufifacies* could be considered to be a beneficial biological control agent for *C. megacephala* (using the latter species as an alternative food source) when both occurred in the same larval media. However, our results indicated a strong positive relationship between the trap catch of *C. megacephala* and *C. rufifacies*, suggesting similar host preference and environmental tolerance between them. Moreover, *C. megacephala* is historically sympatric with *C. rufifacies* and perhaps relatively resistant to predation by *C. rufifacies* (Wells & Kurahashi 1997; Shiao & Yeh 2008), having a competitive advantage over other vulnerable calliphorids. Consequently, larval *C. rufifacies* might not be suitable for use as a biological control agent of *C. megacephala* under natural conditions in Thailand. Further research on the factors underlying predation in this species is warranted.

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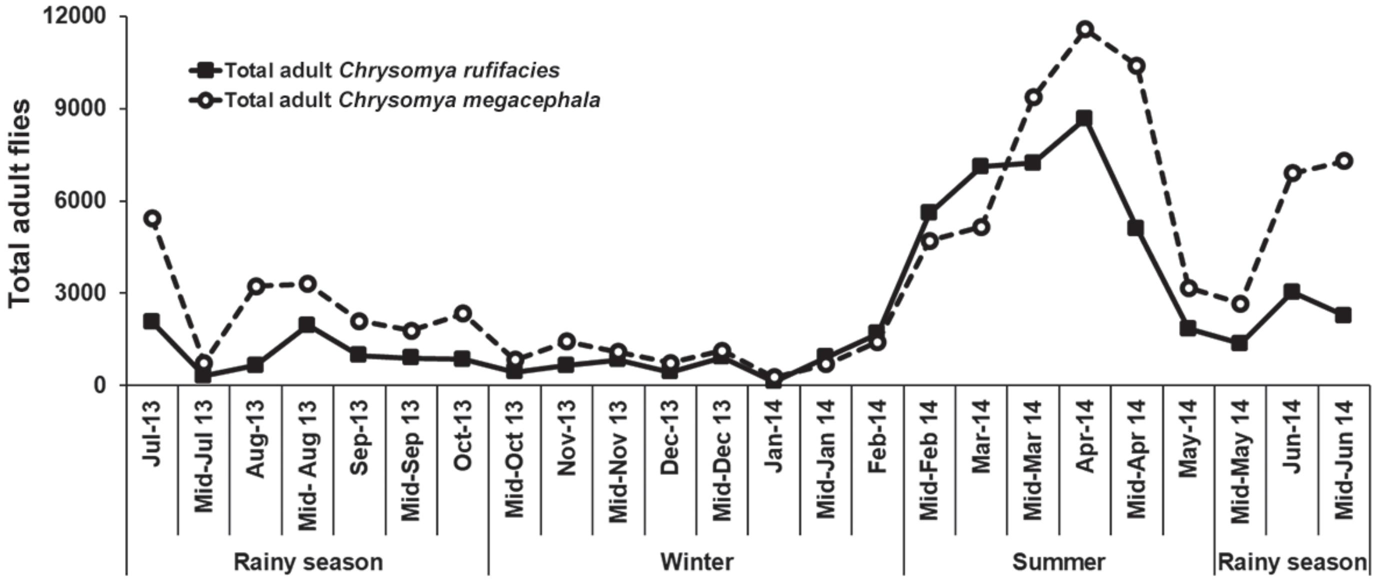

Fig. 2. Seasonal fluctuation of total adult *Chrysomya rufifacies* and *C. megacephala* collected in Chiang Mai Province, northern Thailand during Jul 2013 to Jun 2014, representing a strong positive correlation between both species (r = 0.911; $P < 0.001$).

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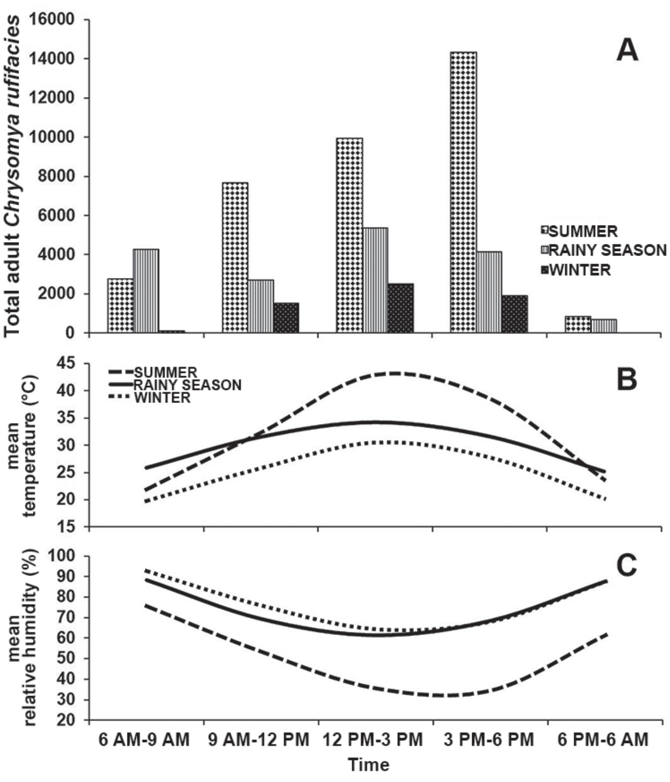

Fig. 3. (A) Daily activity pattern of total adult *Chrysomya rufifacies* collected in each season; (B) Mean temperature; and (C) Mean relative humidity recorded at the study sites for each season in Chiang Mai Province, northern Thailand during Jul 2013 to Jun 2014.

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