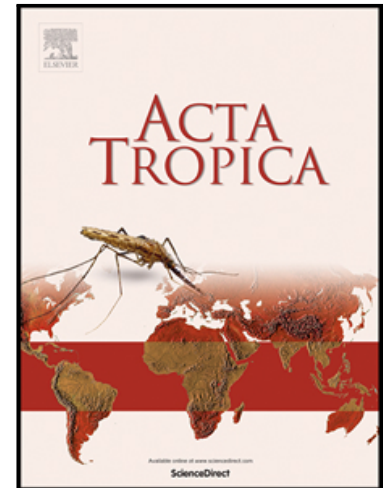


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Daily and seasonal variation of muscid flies (Diptera: Muscidae) in Chiang Mai province, northern Thailand

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## Highlights

- Muscid flies are of medical and veterinary importance worldwide.
- Understanding flight activity and fluctuations of muscid fly abundances as related to climatic factors will be useful for both designing laboratory-rearing systems and establishing effective control programs.
- Our study demonstrates daily flight activity and seasonal abundance of muscid flies in relation to relative humidity and temperature varied among species and microhabitats.

Revised

Acta Tropica

Original paper

**Daily and seasonal variation of muscid flies (Diptera: Muscidae) in Chiang Mai province, northern Thailand**

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## ABSTRACT

Flies of the family Muscidae, or muscids, are of medical and veterinary importance worldwide due to their recognition as nuisance pests and myiasis-producing agents. Effective control of muscids requires biological information on population dynamics daily and across seasons. In this study, such patterns were investigated in three different microhabitats (e.g., forest area, palm plantation and longan orchard) in a suburban area of Chiang Mai Province, northern Thailand. Adult fly samplings were conducted for 24-h intervals using semi-automatic traps and 1-day old beef offal as bait. Samplings were carried out twice per month from July 2013 to June 2014. A total of 3,419 muscids were trapped, comprising nine species, with *Musca domestica* Linnaeus accounting for the majority ( $n = 1,329$ ; 38.9%) followed by *Hydrotaea spinigera* Stein ( $n = 770$ ; 22.5%) and *Musca ventrosa* Wiedemann ( $n = 740$ ; 21.7%). The greatest overall abundance was in the longan orchard location ( $n = 1,508$ ; 44.1%). Community structure peaked during the rainy season (mid-May to mid-Oct). Peak activity during the day was late morning (9.00 to 12.00 h) for *M. domestica*, early morning (6.00 to 9.00 h) for *H. spinigera*, and early afternoon (12.00 to 15.00 h) for *M. ventrosa*. Temperature had no significant effect on the abundance of *M. domestica* ( $r_s = -0.030$ ,  $p = 0.576$ ) or *H. spinigera* ( $r_s = 0.068$ ,  $p = 0.200$ ), but had a weak negative correlation with *M. ventrosa* ( $r_s = -0.238$ ,  $p = 0.0001$ ). Relative humidity had a weak negative correlation with *M. domestica* ( $r_s = -0.263$ ,  $p = 0.0001$ ), *H. spinigera* ( $r_s = -0.107$ ,  $p = 0.043$ ) and *M. ventrosa* ( $r_s = -0.344$ ,  $p = 0.0001$ ). More females ( $n = 2,078$ ) were trapped than males ( $n = 761$ ). These

results provide baseline information of daily and seasonal dynamic activity of muscid flies under natural conditions, which is the prerequisite information for effective control measures.

**Keywords:** *Musca*, *Hydrotaea*, House fly, Flight activity, Control

## 1. Introduction

The Muscidae, filth flies or muscids, is one of the most diverse groups within Diptera with more than 100 species reported in Thailand (Tumrasvin and Shinonaga, 1978, 1982). Muscid flies include non-biting and hematophagous species of medical and veterinary pests (Byford et al., 1992; Malik et al., 2007; Baldacchino et al., 2013). Some non-biting muscids, such as the house fly, *Musca domestica*, Linnaeus, and the bazaar fly or eye-seeking fly, *Musca sorbens* Wiedemann, are mechanical vectors of several pathogenic microorganisms (Sukontason et al., 2007a; Khamesipour et al., 2018; Pohlenz et al., 2018). At least 130 pathogens, including bacteria, fungi, virus, protozoa, and helminth eggs, have been isolated from *M. domestica* (Khamesipour et al., 2018). Furthermore, *M. domestica* is an ectoparasite that can cause facultative myiasis in humans (Derraik et al., 2010; Abosdera and Morsy, 2013). In forensic investigations, many cases reported muscids associated with the death scenes, e.g., *M. domestica*, *Hydrotaea spinigera* Stein and *Synthesiomyia nudiseta* (van der Wulp) (Sukontason et al., 2007b; Sanford 2017). However, muscid flies have received little interest in forensic entomology research due to taxonomic limitations regarding their identification (Grzywacz et al., 2017).

Although, research on muscids has been conducted on their taxonomy and systematics (Grzywacz et al., 2017; Ren et al., 2018), a majority of publications are focused on their vector potential (Bahrndorff et al., 2017; Haddow et al., 2017), control (Breijjo et al., 2017; Zhang et al., 2017) and ecology (Geden, 2005; Gerry et al., 2011). In Thailand, surveys on

non-biting flies of medical importance have been conducted. However, due to the small proportion of muscids collected, most publications emphasized data on blow flies (Diptera: Calliphoridae) (Moophayak et al., 2014; Klong-klaew et al., 2017). Furthermore, many field studies of muscids in Thailand focused mainly on the hematophagous species, such as the stable fly, *Stomoxys calcitrans* (Linnaeus) (Keawrayup et al., 2012; Phasuk et al., 2013). Very little information on seasonal abundance and daily flight activity of non-biting muscids has been reported (Sucharit and Tumrasvin, 1981a; Ngoen-klan et al., 2011) regardless of their important roles as mechanical carriers of several pathogens to humans (Sukontason et al., 2007a; Chaiwong et al., 2014). Although *M. domestica* exists synanthropically (Ngoen-klan et al., 2011; Chaiwong et al., 2014), its habitat preferences are quite diverse. For instance, in Thailand, high abundance of this species was reported in residential areas and mixed orchard (Ngoen-klan et al., 2011), while small number in paddy fields (Chaiwong et al., 2014). In Malaysia, *H. spinigera* was found associated with rabbit carcasses placed in the jungle, rural area, as well as a highland (Silahuddin et al., 2015). Because of their role as a nuisance, many experiments were conducted near residential areas or potential breeding sites, such as markets, garbage piles, or cattle feedlots (Sucharit and Tumrasvin, 1981b; Chaiwong et al., 2012; Godwin et al., 2018). To our knowledge, only two studies have been set in forests or agricultural areas (Ngoen-klan et al., 2011; Chaiwong et al., 2014).

To establish effective control measures, understanding their population dynamics are necessary (Imai, 1984). The objective of this study was to assess muscids community structure year-round at three different microhabitats (i.e., forest area, longan orchard and palm plantation) as related to temperature and relative humidity.

## 2. Materials and methods

## 2.1. Study area

The study area was located at Mae Hia Agricultural Research, Demonstration and Training Center of Mueang Chiang Mai district, Chiang Mai Province, northern Thailand (Fig. 1). The study sites were selected to represent two different settings, (1) agriculture areas [a longan orchard (N18°45'56.66", E98°55'40.13"), and a palm plantation (N18°45'27.841", E98°55'48.515")] and (2) a forest area (N18°46'01.08", E98°56'08.3"). These study sites were located near cattle feedlots, which were assumed to have high number of muscid flies (Tumrasvin and Shinonaga, 1977; Ngoen-klan et al., 2011). Each study site was located ~1.5 km from each other (Klong-klaew et al. 2017). The forested area was covered with teak (*Tectona grandis* L.f.) and various annual plants (i.e., *Mimosa pudica* L.) of mixed deciduous forest. In Longan orchard, the major crop was *Dimocarpus longan* Lour. (Sapindaceae). Palm plantation was full of *Tenera* palm trees (*Elaeis guineensis* Jacq.).

## 2.2. Adult fly collection

A semi-automatic funnel trapping system was used for collecting adult flies commonly associated with decomposition (Klong-klaew et al., 2017). The trap consists of five basic parts: 1) external metal case (40×40×60 cm), 2) fly net (36×36×85 cm), 3) fly entrance module made of transparent plastic board, 4) timer, and 5) CD-player tray (Fig. 2). The trap is set to operate at specific time (Table 1) by timer. At the time of initiating trapping, a timer triggers the releasing of electric power to supply a CD-player tray. A CD-player tray slide from the slot loading drive which is connected to a fly entrance module by a string, then it pulls the string and the entrance module is lifted up allowing flying insects to enter to luring bait. At the set time, the tray slides back into the loading slot releases the string. The fly entrance module slides down and closes a fly entrance. All trapped flies were held in the fly

net. The trap was manually emptied by removing the fly net and installing a new one for the next experimental loop (Klong-klaew et al., 2017).

Adult flies were sampled on 24 hour intervals every two weeks from July 2013 to June 2014. Five semi-automatic traps were operated at five different time intervals (Table 1) over a 24 h cycle to determine periodic activity of flies, resulting continuously operated setting. In each experiment, four day traps (early morning, late morning, early afternoon, and late afternoon) and one night trap were installed along a transect with 50 m between consecutive traps (Klong-klaew et al., 2017).

Each trap was baited with 300 g of 1-day old beef offal from the same butcher shop throughout the year. Material was purchased and prepared as needed (Table 1). Bait at room temperature was inserted into traps which were then placed in the field at 6.00, 9.00, 12.00, 15.00, and 18.00 h (Klong-klaew et al., 2017).

Each semi-automatic trap was placed inside a wire cage (65×55×90 cm) covering with a transparent plastic sheet on top to prevent scavenger and rain damage. Furthermore, the traps were placed inside transparent plastic trays filled with water to prevent ants and other crawling insects from accessing and contaminating the bait. Hourly temperature (°C) and relative humidity, RH (%) were recorded at the study sites using data loggers (Ebro EBI 20-TH1; Ebro Electronic GmbH & Co. KG, Ingolstadt, Germany).

All traps were allowed to collect insects for 24 h. Traps were then transferred the fly net to the Department of Parasitology, Faculty of Medicine, Chiang Mai University. Flies within the traps were sacrificed by freezing traps at -20 °C for 2 h. Muscids were identified to species using taxonomic keys (Tumrasvin and Shinonaga, 1978, 1982), sexed and counted under a dissecting microscope (model SZ2-ILST, Olympus Corporation, Tokyo, Japan).

### 2.3. Statistical analysis



To determine the relationship of environmental variables (temperature and RH) on number of flies collected, a bivariate correlation analysis and Spearman's rank correlation coefficient ( $r_s$ ) were employed. To study daily activity, trapped flies in each microhabitat were combined, and the totals were analyzed by using Kruskal-Wallis test followed by Mann Whitney  $U$ -test:  $p < 0.05$ . Statistical analysis was performed using IBM SPSS Statistics for Windows, version 22.0 ( $\alpha = 0.05$ ) (IBM Corp., Armonk, NY, USA) and JMP<sup>®</sup>, version 14 (SAS Institute Inc., Cary, NC, USA). The diversity of flies collected from each study sites was detected using Shannon-Wiener diversity index (H) and Simpson's diversity index (1-D). The indices were calculated using PAST program (version 3.2) (Hammer et al., 2001).

### 3. Results

Between July 2013 to June 2014, 3,419 flies comprising nine species of non-biting muscids were collected from the three study sites. These flies included *Musca*, *Hydrotaea*, *Dichaetomyia* and *Synthesiomyia* spp. (Table 2). Among them, *M. domestica* was the most abundant totaling 38.9% ( $n = 1,329$ ), followed by *H. spinigera* ( $n = 770$ ; 22.5%) and *M. ventrosa* ( $n = 740$ ; 21.7%). The other muscids, which were small number (<10% each), were omitted from statistical analysis. This paper, therefore, would focus on the three most abundant species, namely *M. domestica*, *H. spinigera* and *M. ventrosa*.

The overall muscids abundance was higher in longan orchard ( $n = 1,508$ ; 44.1%) than the forest area ( $n = 1,020$ ; 29.9%) and palm plantation ( $n = 888$ ; 26.0%) (Table 2). However, specimens collected in the forest area appeared to be more diverse than in the palm plantation and longan orchard as indicated by the indices of diversity (Table 2).

The variations of seasonal abundance of the three most abundance muscid species were determined during three different climatic seasons: summer (mid-February to mid-May),

rainy season (mid-May to mid-October) and winter (mid-October to mid-February). Flies were more abundant in the rainy season (Table 3, Fig. 3). A total of 85.5% of *M. domestica* was trapped in the rainy season ( $n = 1,137$ ), followed by summer ( $n = 179$ ; 13.5%) and winter ( $n = 13$ ; 1.0%). The fluctuation pattern of this species varied seasonally, peaking in July 2013 (rainy season), then it declined gradually and almost absent at late rainy season. Fly number was consistently low from late rainy season throughout winter and summer, and then it dramatically increased in June 2014 (rainy season) (Fig. 3). Similarly, the greatest number of *M. ventrosa* was trapped in rainy season ( $n = 461$ ; 62.3%). Lower numbers were collected in summer ( $n = 216$ ; 29.2%) and winter ( $n = 63$ ; 8.5%). The fluctuation of *M. ventrosa*, which had a major peak was found in July 2013 (rainy season), and it decreased gradually during winter. Then, it gradually increased during late of winter (February 2014) resulting in a minor peak in late summer (April 2014) (Fig. 3). Likewise, *H. spinigera* numbers peaked during the rainy season ( $n = 552$ ; 71.7%) and were lowest during the summer ( $n = 113$ ; 14.7%) and winter ( $n = 105$ ; 13.6%). Peak trap catch was found in mid-August (rainy season) and the fluctuations were found throughout the year (Fig. 3).

During the one-year survey, more females were trapped than males, resulting in the overall sex ratio (male:female) of: 0.30:1 in *M. domestica*, 0.30:1 in *M. ventrosa*, and 0.57:1 in *H. spinigera* (Table 3).

Temperature, when flies were collected, ranged between 13.7-51.5 °C; RH ranged between 13.7-96.2% (Table 2, Fig. 4). High number of *M. domestica* was trapped at 25-30 °C and 50-80% RH (Fig. 4A), similar to high trap catch of *M. ventrosa* at 25-30 °C and 60-80% RH (Fig. 4B). In contrast, high number of *H. spinigera* was found at 20-25 °C with low RH (<40%) (Fig. 4C). According to statistical analysis, a weak negative correlation between temperature and trap catch of *M. ventrosa* was found ( $r_s = -0.238$ ,  $p = 0.0001$ ). On the contrary, temperature show no significant relationship with trap catches of *M. domestica* ( $r_s =$

-0.030,  $p=0.576$ ) and *H. spinigera* ( $r_s = 0.068$ ,  $p = 0.200$ ). Relative humidity was weak negatively correlated with trap catches of *M. domestica* ( $r_s = -0.263$ ,  $p = 0.0001$ ), *M. ventrosa* ( $r_s = -0.344$ ,  $p = 0.0001$ ) and *H. spinigera* ( $r_s = -0.107$ ,  $p = 0.043$ ) (Table 4).

Diurnal activity patterns of *M. domestica*, *M. ventrosa* and *H. spinigera* are presented in Figure 5. Main peak activity of *M. domestica* was found in the late morning (9.00 to 12.00 h). No significant difference was found among day traps, but significant lower number in the night trap (18.00 to 6.00 h) (Fig. 5A). For *M. ventrosa*, peak activity was found in the early afternoon (12.00 to 15.00 h). Only small numbers of flies were collected in the early morning (6.00 to 9.00 h) and at night (18.00 to 6.00 h) (Fig. 5B). In contrast, peak number of *H. spinigera* occurred in the early morning (6.00 to 9.00 h); however, no significant difference was found among day traps. This species showed a constant activity along the day with a significant lower number trapped by the night trap (18.00 to 6.00 h) (Fig. 5C).

#### 4. Discussion

To develop integrated pest management strategies for a targeted species, monitoring its population dynamics is necessary to determine when to apply various control methods and obtain the most effective consequences (Imai, 1984). As a result of the 3,419 specimens collected year-round, this study presents the diversity of muscid flies in three different microhabitats focusing on comprehensive seasonal and daily flight activity of the three most abundant species; namely *M. domestica*, *M. ventrosa* and *H. spinigera*. Although many studies have reported on the seasonal and daily activity of *M. domestica* (Sucharit and Tumrasvin, 1981a; Ngoen-klan et al., 2011; Godwin et al., 2018), this was the first to elucidate the activity of *M. ventrosa* and *H. spinigera*.

The overall abundance of muscids was greatest in longan orchard than forest area and palm plantation. In longan orchard, many flowers and ripe fruits may attract adult flies, which require carbohydrate from these sources (Ngoen-klan et al., 2011). This result was consistent with a previous study in northern Thailand, which reported high abundance of *M. domestica* in residential areas and mixed orchards (Ngoen-klan et al., 2011). By contrast, our muscid results differed from those determined for blow flies (Klong-klaew et al., 2017, 2018; Sontigun et al., 2018). This difference may indicate variation in habitat preference between muscids and blow flies. Regarding the three most common species, *M. domestica*, *H. spinigera* and *M. ventrosa*, low population densities were found during the flowering season in longan orchard (December-February). However, high numbers were found during the fruiting and harvesting season (June-August), indicating that muscids may be attracted and aggregated in longan orchard by the ripeness of fruits rather than the blooming flowers of longan tree.

Peak populations were recorded during the rainy season. This may be due to environmental conditions (mean temperature  $25.4 \pm 0.5$  °C, mean RH  $71.0 \pm 1.6\%$ ) being favorable for muscid flies. A previous study examining fly numbers in cattle feedlots located in Australia also indicated high number of *M. domestica*, particularly after rainfall (Urech et al., 2012). However, our results were in contrast with the study in Australia (Urech et al., 2012; Godwin et al., 2018) and Chile (Figueroa-Roa and Linhares, 2004), where the most abundant flies was reported in warmer months. Ngoen-klan et al. (2011) found peak of *M. domestica* in summer (May) of northern Thailand. These differences may occur due to their study being conducted in variously developed land ranging from city to forest, within three districts of Chiang Mai Province including urban and suburban areas (Ngoen-klan et al., 2011), while our study only limited in the forest and agricultural sites in the suburban area. This may highlight that although climatic factors have impact on the seasonal fluctuations of

flies, the variation can occur among study localities (Jin and Jaal, 2009). Investigation on seasonal abundance of flies should be conducted in several regions to get information for the implement of effective fly control program in a given area.

Temperature and RH seemed to impact fluctuations of muscids throughout the various seasons. However, statistical analysis indicated temperature had no significant effect on the abundance of *M. domestica* and *H. spinigera* and had a weak negative correlation with *M. ventrosa*. This is in contrast with a previous study of *M. domestica* in northern Thailand where a positive correlation was reported (Ngoen-klan et al., 2011). Temperature has been suggested to significantly effect locomotor activity of *M. domestica* with activity being hampered when temperature were  $>35^{\circ}\text{C}$  (Schou et al., 2013). Our results indicated *H. spinigera* had distinct peak in abundance in lower temperature ( $20\text{--}25^{\circ}\text{C}$ ) than *M. domestica* and *M. ventrosa* ( $25\text{--}30^{\circ}\text{C}$ ). This is consistent with the study of *M. domestica* in poultry farms in Malaysia which reported house flies were most abundant and active at warm temperature ( $25\text{--}35^{\circ}\text{C}$ ) (Jin and Jaal, 2009).

Muscidae for the most part is diurnal (Dakshinamurty, 1948; Sucharit and Tumrasvin, 1981a). In the current study, peak in flight activity appeared at different times of the day for different fly species. *Musca domestica* peaked during late morning (9.00 to 12.00 h), *H. spinigera* during early morning (6.00 to 9.00 h) and *M. ventrosa* during early afternoon (12.00 to 15.00 h). Only a small number was trapped by night time trap. Previous study in Bangkok, central Thailand, indicated bimodal peak activity of *M. domestica*, with a major peak at 12.00 to 14.00 h and a minor peak at 8.00 to 10.00 h (Sucharit and Tumrasvin, 1981a). Light intensity plays an important role in flight activity of flies; therefore, they were active during sunrise to sunset (Dakshinamurty, 1948; Sucharit and Tumrasvin, 1981a). However, light intensity was not measured throughout this study. In Malaysia, *M. ventrosa* was a strictly diurnal species, while *M. domestica* exhibited both diurnal and nocturnal

activities (Nazni et al., 2007). However, their nocturnal traps settings were 19.00 to 7.00 h, which was different from our studies. Furthermore, the duration between sunrise and sunset (daylight hour) was different depending on locality. In this regard, the variation of daylight hours should be considered when study daily flight activities of muscid flies in each locality.

A greater proportion of female *M. domestica* rather than males were collected in the current study, which was also documented previously (Ngoen-klan et al., 2011; Mulieri et al., 2015; Klong-klaew et al., 2018; Sontigun et al., 2018). Muscid females may be seeking beef-offal bait as protein sources to develop eggs or use as breeding places for their offspring (da Costa and Mendes, 2014; Upakut et al., 2017). Males visiting bait traps are most likely seeking mates (Mulieri et al., 2015). Although our results indicate variation in sex-ratio was partially due to season (Table 3), no general conclusion can be drawn from this finding in a limited number of locality study.

Little information pertaining to *H. spinigera* and *M. ventrosa* has been reported previously (Pont, 1973; Nazni et al., 2007; Heo et al., 2015). Even though only few were collected from our study, they potentially could serve as vectors for a number of pathogens as is *M. domestica* due to their propensity to feed on human feces and animal dung, as well as their association with human and domestic animal dwellings (Pont, 1973; Magpayo et al., 1987; Heo et al., 2015).

From a forensic perspective, *M. domestica* and *H. spinigera* are important as they are commonly involved with human decomposition in Thailand (Sukontason et al., 2007b) and the USA (Sanford, 2017). Additionally, they had been reared and collected from animal carcasses in Australia (Pont, 1973) and Malaysia (Chen et al., 2010; Silahuddin et al., 2015). These species are found across diverse habitats ranging from urban to forested areas (Ngoen-klan et al., 2011; Silahuddin et al., 2015); therefore, our results may expand knowledge

pertaining to their biology in natural conditions which could be applied in forensic context (e.g., determining time and season of the death).

In conclusion, our study provides greater detail of the diversity of muscid flies in three different microhabitats (e.g., forest area, longan orchard and palm plantation). The daily and seasonal activity of the three most abundant species, *M. domestica*, *M. ventrosa*, and *H. spinigera* from a year-round trapping, has been reported. Peak activity appeared at different times for each species. Our study provides an insight into flight activity, fluctuations of fly abundances, and climatic factor affecting the abundance, which will be useful for designing laboratory rearing systems (i.e., optimal temperature and RH) for these species and can be useful for establishing effective control programs. Information obtained from this study not only benefits for Thailand, but can be baseline information for other countries where these species distributed.

#### **Author statement**

This manuscript (ACTROP\_2019\_1653) entitled “Daily and seasonal variation of muscid flies (Diptera: Muscidae) in Chiang Mai province, northern Thailand” has not been submitted for consideration elsewhere.

All authors approved this submission.

#### **Conflict of interest**

There is no conflict of interest for this manuscript.

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**Table 1**

Semi-automatic funnel trap operation times in each experimental circle.

	Trap	Trap periodicity	Open	Closed	Duration (hour)
Day trap	1	Early morning	6.00	9.00	3
	2	Late morning	9.00	12.00	3
	3	Early afternoon	12.00	15.00	3
	4	Late afternoon	15.00	18.00	3
Night trap	5	Night	18.00	6.00	12

**Table 2**

Relative abundance of muscid flies collected from three habitats during July 2013-June 2014.

Variable	Study sites			Total number (%)
	Forest area	Longan orchard	Palm plantation	
<b>Climatic variable*</b>				
Temperature (°C)	27.7 (13.7-51.5)	27.4 (13.7-50.4)	27.4 (13.7-50.4)	
Relative humidity (%)	75.1 (20.7-96.2)	72.8 (20.7-96.2)	61.7 (13.7-94.8)	
<b>Diversity indices</b>				
Simpson (1-D)	0.79	0.62	0.73	
Shannon (H)	1.76	1.32	1.51	
Taxa	9	8	9	
<b>Lists of species</b>				
<i>Musca domestica</i> Linnaeus	232	858	239	1,329 (38.9)
<i>Musca ventrosa</i> Wiedemann	222	308	210	740 (21.7)
<i>Musca pattoni</i> Austen	95	41	13	149 (4.4)
<i>Musca conducens</i> Walker	19	0	5	24 (0.7)
<i>Hydrotaea spinigera</i> Stein	308	136	326	770 (22.5)
<i>Hydrotaea chalcogaster</i> (Wiedemann)	26	59	52	137 (4.0)
<i>Hydrotaea leucostoma</i> (Wiedemann)	11	13	15	39 (1.1)
<i>Dichaetomyia quadrata</i> (Wiedemann)	82	86	5	173 (5.1)
<i>Synthesiomyia nudiseta</i> (van der Wulp)	25	7	23	55 (1.6)
Total (%)	1,020 (29.9)	1,508 (44.1)	888 (26.0)	3,416 (100)

\* Median (min-max)

**Table 3**

Muscidae flies collected by semi-automatic traps from July 2013-June 2014.

Season*	<i>M. domestica</i>			<i>M. ventrosa</i>			<i>H. spinigera</i>		
	Male	Female	M/F	Male	Female	M/F	Male	Female	M/F
Summer	38	141	0.27	27	189	0.14	50	63	0.79
Rainy season	269	868	0.31	126	335	0.38	195	357	0.55
Winter	1	12	0.08	19	44	0.43	36	69	0.52
Total	308	1,021	0.30	172	568	0.30	281	489	0.57

\*Summer: mid-Feb to mid-May, Rainy season: mid-May to mid-Oct, Winter: mid-Oct to mid-Feb. M/F = sex ratio

**Table 4**

Correlation coefficient between the climatic factors and fly populations collected during Jul 2013-Jun 2014.

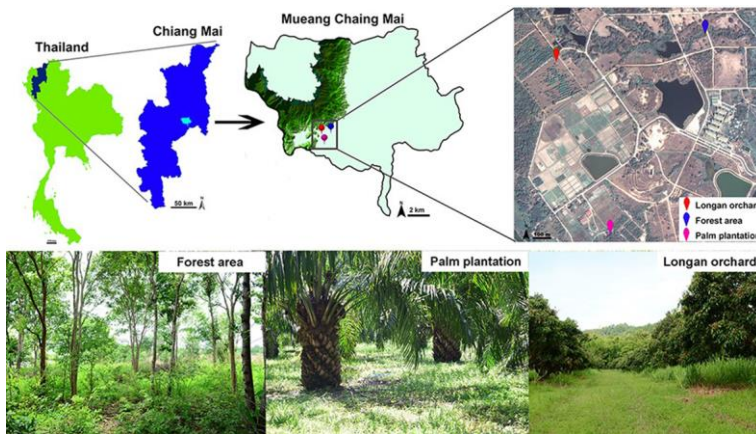
Climatic factors	Fly species			
		<i>Musca domestica</i>	<i>Musca ventrosa</i>	<i>Hydrotaea spinigera</i>
Temperature	$r_s$	-0.030	-0.238**	0.068
	$p$	0.576	0.0001	0.200
Relative humidity	$r_s$	-0.263**	-0.344**	-0.107*
	$p$	0.0001	0.0001	0.043

The values are presented as Spearman's rank correlation coefficient ( $r_s$ ) ( $p$ -sig, two-tailed).

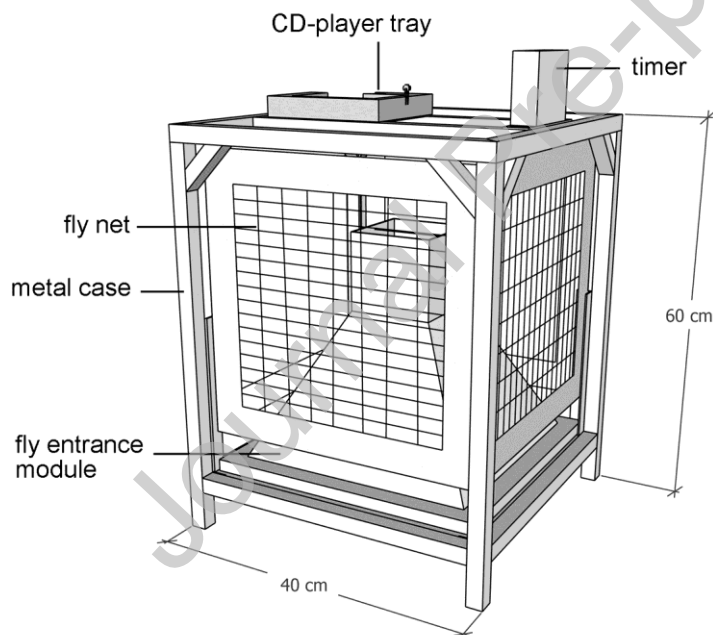
\*  $p = 0.05$ , \*\*  $p = 0.01$ , significant correlations.



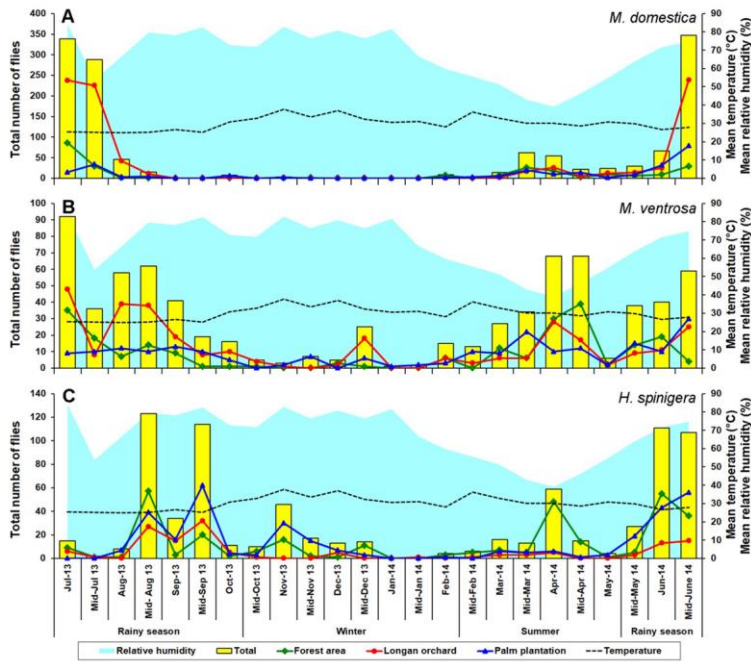
## Figure legends



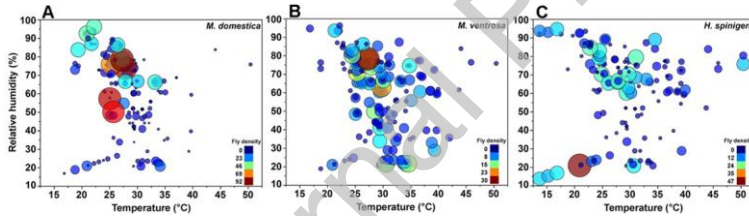
**Fig. 1.** A map of Thailand showing the study sites located in Mueang Chiang Mai district of Chiang Mai Province, northern Thailand. Satellite imagery of three sampling locations including forest area, longan orchard and palm plantation.



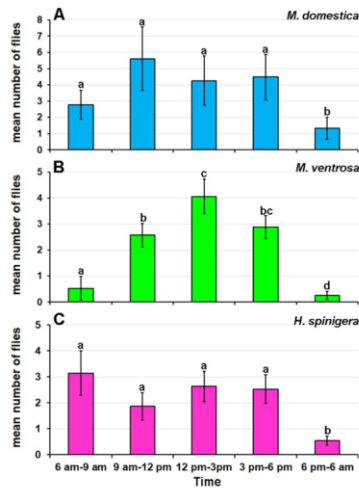
**Fig. 2.** Diagram of semi-automatic funnel trap.



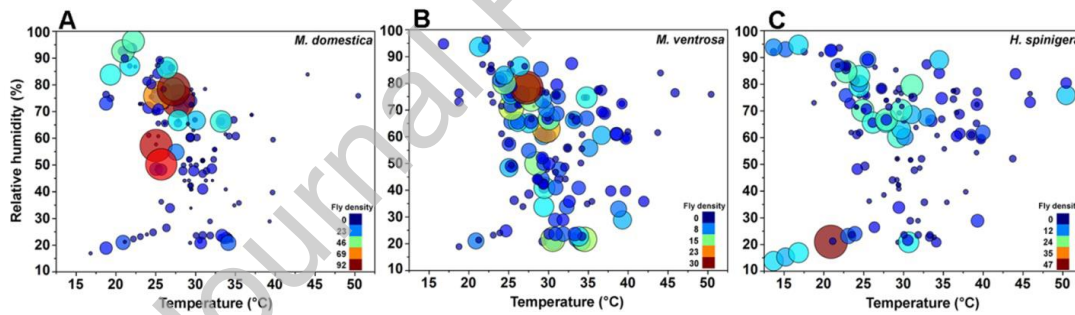
**Fig. 3.** Monthly fluctuations in total population density of *M. domestica* (A), *M. ventrosa* (B) and *H. spinigera* (C) trapped in each study site during Jul 2013-Jun 2014, and annual variation of temperature and relative humidity recorded during the fly survey.



**Fig. 4.** The number of captured flies; *M. domestica* (A), *M. ventrosa* (B) and *H. spinigera* (C), in relation to study site temperature (°C) and RH (%). Each bubble represents fly density in each temperature and RH; a bigger size of bubble symbolizes a greater number of captured flies; navy blue bubbles indicate the lowest density and red bubbles symbolize the highest density of flies.



**Fig. 5.** Annual activity of the most abundant Muscidae species collected in a one-year survey; *M. domestica* (A), *M. ventrosa* (B) and *H. spinigera* (C) [mean number of individuals per trap  $\pm$  standard error]. The different letter represents a significant difference (Mann-Whitney *U* test;  $p < 0.05$ ).



**Graphical Abstract**