



## Research article

# Comparison of stable fly (Diptera: Muscidae) population dynamics on a cattle farm and at an open zoo in Thailand

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## Abstract

*Stomoxys* flies (Diptera: Muscidae) are an important animal pest and a potential vector of pathogens. The density and abundance of these flies vary depending upon the type of ecological habitat. This study compared the diversity and abundance of *Stomoxys* flies in two different ecological habitats: a small-scale cattle farm and a zoological park. Vavoua traps were used to capture adult flies during 0600–1800 hours for two consecutive days every month, alternating between sites for 2 yr. Among the six species of *Stomoxys* spp. described in Thailand, the study sites had four that were morphologically identified as *Stomoxys calcitrans* (81.1%), *S. indicus* (17.8%), *S. sitiens* (0.6%) and *S. uruma* (0.5%). The cosmopolitan *S. calcitrans* was the most abundant at both sites. A greater number of flies were captured on the farm (8,758 specimens) than at the zoo (1,416 specimens). A seasonal effect was observed at the farm, with abundance peaking in August in the rainy season ( $p < 0.05$ ). Overall, the number of *Stomoxys* flies trapped peaked during 1400–1800 hours on the cattle farm and during 1400–1600 hours at the zoo. On the farm, there was a significantly ( $p < 0.05$ ) higher number of *Stomoxys* flies during the rainy season (July–October) than in other seasons. A clear understanding of habitat preferences by *Stomoxys* flies with respect to species diversity, abundance and daily activity patterns will facilitate and improve the efficacy of fly prevention and control strategies in Thailand.

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## Introduction

Stable flies (Diptera: Muscidae, genus *Stomoxys*) are pests of livestock and wild animals in many parts of the world (Zumpt, 1973; Mullens et al., 1988; Kunz et al., 1991; Baldacchino et al., 2013). Among the 18 species in the subfamily Stomoxyinae, only one species, *Stomoxys calcitrans* (L. 1758), is cosmopolitan. In addition to *S. calcitrans*, several other Stomoxyine flies are also considered pests of animals, including *S. niger*, *S. sitiens* and *S. indicus* (Wall and Shearer, 1997).

Both sexes feed on blood, are aggressive and sometimes attack humans (Baldacchino et al., 2013). Stomoxyinae flies breed in animal dung and organic waste and in the case of *Stomoxys* most prolifically in elephant, buffalo and cattle dung (Jeanbourquin and Guerin, 2007). Considerable economic impact on the beef and dairy industries was reported in the United States (Taylor et al., 2012). Stable flies are also known to be mechanical vectors of many different disease pathogens (Baldacchino et al., 2013; Malaithong et al., 2019).

Populations of adult stable flies can be assessed using several different methods. Many studies have used direct counts or collections from animals (McNeal and Campbell, 1981; Berry and Campbell, 1985). The more field-friendly Vavoua trap (named after a village in Africa) has been used for stable fly collection; it is made from blue fabric according to the design previously described by Laveissière and Grebaut (1990). This trap has proved very efficient at capturing *Stomoxys* spp. in many African countries (Holloway and Phelps, 1991; Mihok et al., 1995, 1996), on Réunion island (Gilles et al., 2007) and in some parts of Thailand (Malaithong et al., 2019). Comprehensive studies on stable flies (essentially on *S. calcitrans*) have been documented in several areas throughout the world (Taylor et al., 2007; Bitome-Essono et al., 2015; Sharif et al., 2019). Species identification, seasonal abundance and daily activity patterns of Stomoxyine flies have been studied in Thailand (Malaithong et al., 2019) over the last decade. The present survey compared the stable fly populations of two potential habitats, a small-scale beef cattle farm and a zoo park, in terms of population dynamics and potential factors influencing their abundance.

## Materials and Methods

### Study sites

The two sites selected for the field experiment were a small beef cattle farm (BCF) and a zoo (KKOZ) as follow:

#### 1. Beef cattle farm

This farm is located in Moo 9 of Thai Samakkee subdistrict, Wang Nam Khiao district, Nakhon Ratchasima province (N14°22'1.3", E101°55'31.6"), approximately 200 km northeast of Bangkok. The site was 3 km away from Tub Lan National Park, one of the largest national parks in Northeastern Thailand. Approximately 20 head of cattle are kept on this farm on 1.21 ha. No insecticide was used to protect the cattle from insect bites.

#### 2. Khao Kheow Open Zoo (KKOZ)

KKOZ is situated at 235 Moo 7, Bangpra subdistrict, Sriracha district, Chonburi province, approximately 150 km east of Bangkok (N13°12'56.2", E101°3'14.3"). The area has been established as a unit of the Zoological Park Organization of Thailand and has been open to the public since 1978. In 1984, the Zoological Park Organization allowed an expansion of the area to 4.046 km<sup>2</sup> for wildlife conservation and for the care of injured animals and wildlife. Today, KKOZ has about 3,000 animals belonging to 250 species, distributed over 8.09 km<sup>2</sup>.

### Trapping design

At each collection site, flies were caught using Vavoua traps (Laveissière and Grebaut, 1990). On BCF, 10 Vavoua traps were set up along a fence, approximately 10 m apart, and left operational overnight before the first collection at 0600 hours. Collections were made every 2 hr during 0600–1800 hours over two consecutive days every other month (alternating with KKOZ) for 2 yr. Ambient air temperature and relative humidity were recorded every 2 hr over the 2 d throughout the study period. Monthly rainfall data were obtained from the Wang Nam Khiao District Meteorological Station located approximately 2 km from the study site.

At KKOZ, 14 Vavoua traps were set up to cover potential sites for stable flies. All traps were placed close to animal houses throughout the zoo. The traps were left operational during the night before the first collection at 0600 hours. Adult stable flies in the Vavoua traps were collected every 2 hr during 0600–1800 hours. Collections were made for two consecutive days every other month for 2 yr. Ambient air temperature and relative humidity were recorded every 2 hr throughout every two-day collection period and rainfall data were obtained from the local meteorological station located in the zoo.

At both sites, all the flies collected were preserved in 95% ethanol and recorded by trap, date, and hour of collection. Subsequently, specimens were brought to the Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand, for morphological identification following Zumpt (1973).

#### Data analysis

Normality of data was checked using the Shapiro-Wilk test. Normally distributed data on the total number of stable flies collected per trap were subjected to analysis of variance, followed by a multiple comparison test of means using the least significance difference (LSD) test. For data that did not follow a normal distribution (such as the number of flies collected per hour), the Kruskal-Wallis test was used followed by a multiple comparison test (using the `kruskalmc` function of the `pgrmness` package in Giraudoux (2018) to identify significant differences in fly numbers among categories of collection times. The Kruskal-Wallis test was performed using the LSD method. The effects of environmental factors (ambient temperature and relative humidity) on fly abundance were analyzed using a generalized linear model (GLM). A  $\chi^2$  test was used to determine significant differences, with  $p$  values set at  $< 0.05$ . All data were analyzed using RStudio version 3.3.3 (RStudio Team, 2019) and the R software (R Core Team, 2018) with the packages `pgrmness` (Giraudoux, 2018) and `agricolae` (Mendiburu, 2019).

## Results and Discussion

During the 2 yr study period, 10,174 specimens were collected, representing four different species. *Stomoxys calcitrans* was the most abundant species (81.1%), followed by *S. indicus* (17.8%), *S. sitiens* (0.6%) and *S. uruma* (0.5%). In total, 86.1% of the stable flies collected were from BCF and 13.9% from KKOZ.

#### Monthly fly collections

On BCF, 2,048 flies were trapped in August Year-1, and 1,711 in August Year-2. The lowest numbers were observed in April Year-1 (81 specimens) and December Year-1 (201 specimens), as shown in Table 1. The corresponding curves clearly show that abundance peaked in August for both *S. calcitrans* and *S. indicus* (Fig. 1). The total yields from the 10 traps are represented in Fig. 2. Traps number 4 and 3 were the

most efficient, with a total of 1,959 flies (22.4%) in trap number 4. Overall, *S. calcitrans* represented 79.7% of the trapped specimens, *S. indicus* 19.2% and the other two species together accounted for only 1.1%.

At KKOZ, 228 flies were trapped in November Year-1, 387 in March Year-2 and only 22 in July Year-2. The corresponding curves display irregular peaks of abundance for *S. calcitrans* (Fig. 3). Overall, *S. calcitrans* represented 90.1% of the collected flies, *S. indicus* 8.6% and the other two species together accounted for only 1.3%. The proportions of flies trapped in each of the 14 traps are indicated on Fig. 4. A significantly higher number of flies was found in trap number 2 (near a herd of female Eld's deer) compared with the other traps.

#### Diurnal activity

The diurnal activity of flies was studied for 12 mth at both sites. Flies were collected at 2 hr intervals throughout each day of trapping, during 0600–1800 hours. On BCF, activity (the number of trapped flies per trap) peaked during 1400–1800 hours (Fig. 5). At KKOZ, there was a slight but not significant increase in diurnal activity during 1400–1600 hours (Fig. 6).

#### Seasonal abundance

Generally, in Thailand, the year can be divided into three different seasons: a hot season from March to June, a rainy season from July to October and a cool season from November to February. Table 2 shows the total numbers of *Stomoxys* flies trapped on BCF and KKOZ recorded during the different seasons over the 2 yr period.

On BCF, males and females of both *S. calcitrans* and *S. indicus* were more abundant during the rainy season (Table 2). No correlation was found during the hot season (March–June) between the number of flies trapped and the temperature, but temperature did have a significant influence on the abundance of flies during the rainy season ( $\chi^2 = 7.12$ ,  $df = 1$ ,  $p = 0.008$ ) and the cool season ( $\chi^2 = 22.65$ ,  $df = 1$ ,  $p < 0.0001$ ); see also Fig. 7.

At KKOZ, there were no significant differences among the numbers of flies trapped per season. In addition, there were few female specimens of *S. calcitrans* and *S. indicus*, which explained why the mean and SD were very similar, indicating poor reliability (Table 2). Regarding the effect of climatic parameters, no significant correlations were found between fly numbers and either temperature or humidity over the duration of the study.

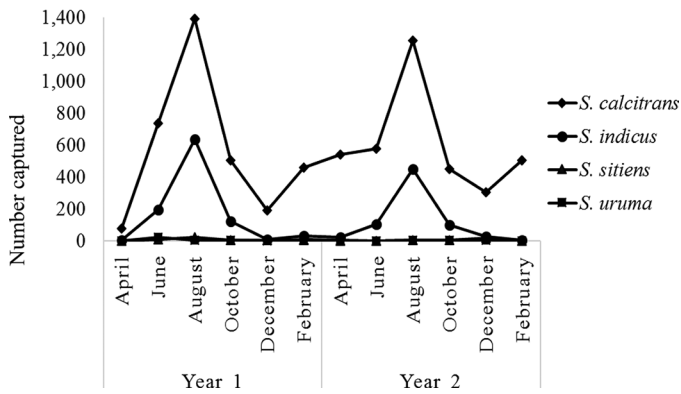
**Table 1** Monthly captures of *Stomoxys* flies using Vavoua traps on a local beef cattle farm and at Khao Kheow Open Zoo

Collection site	Month	Species								Total
		<i>S. calcitrans</i>		<i>S. indicus</i>		<i>S. uruma</i>		<i>S. sitiens</i>		
		Male	Female	Male	Female	Male	Female	Male	Female	
Local beef cattle farm	Yr-1									
	Apr	45	32	1	3	0	0	0	0	81
	Jun	511	225	76	117	10	9	6	2	956
	Aug	980	412	289	344	0	2	8	13	2,048
	Oct	301	204	48	72	0	1	1	1	628
	Dec	108	81	7	2	0	0	2	1	201
	Feb	281	177	11	19	4	1	0	3	496
	Yr-2									
	Apr	305	235	8	12	0	3	0	0	563
	Jun	332	245	51	51	0	0	0	0	679
	Aug	852	403	169	280	3	1	0	3	1,711
	Oct	275	172	41	56	2	0	2	2	550
	Dec	191	112	6	19	1	0	4	10	343
	Feb	341	160	0	1	0	0	0	0	502
	Total	4,522	2,458	707	976	20	17	23	35	8,758
Khao Kheow Open Zoo	Yr-1									
	May	40	20	8	10	3	0	0	2	83
	Jul	35	19	5	5	4	0	0	1	69
	Sep	109	20	11	9	0	0	1	1	151
	Nov	186	17	11	14	0	0	0	0	228
	Jan	52	12	9	3	0	0	0	0	76
	Mar	303	79	2	1	0	0	0	2	387
	Yr-2									
	May	94	20	2	0	0	0	0	0	116
	Jul	14	3	3	2	0	0	0	0	22
	Sep	42	5	6	9	0	0	0	1	63
	Nov	79	10	3	6	3	0	0	0	101
	Jan	39	15	2	0	0	0	0	0	56
	Mar	53	10	0	1	0	0	0	0	64
	Total	1,046	230	62	60	10	0	1	7	1,416

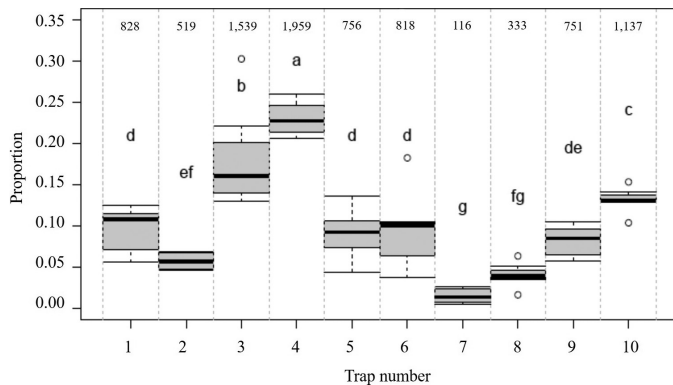
**Table 2** Mean numbers of *Stomoxys* flies on a beef cattle farm and at Khao Kheow Open Zoo caught during three climatic seasons

Collection site	Species	Mar–Jun	Jul–Oct	Nov–Feb
Local beef cattle farm	<i>S. calcitrans</i> male	150±98 <sup>a</sup>	301±175 <sup>a</sup>	115±59 <sup>a</sup>
	<i>S. calcitrans</i> female	92±49 <sup>b</sup>	149±65 <sup>a</sup>	66±26 <sup>b</sup>
	<i>S. indicus</i> male	17±17 <sup>ab</sup>	68±69 <sup>a</sup>	3±3 <sup>b</sup>
	<i>S. indicus</i> female	23±24 <sup>ab</sup>	94±85 <sup>a</sup>	5±5 <sup>b</sup>
	All species	285±175 <sup>ab</sup>	617±364 <sup>a</sup>	193±75 <sup>b</sup>
Khao Kheow Open Zoo	<i>S. calcitrans</i> male	61±57 <sup>a</sup>	25±22 <sup>a</sup>	45±36 <sup>a</sup>
	<i>S. calcitrans</i> female	16±15 <sup>a</sup>	6±5 <sup>a</sup>	7±4 <sup>a</sup>
	<i>S. indicus</i> male	1±2 <sup>a</sup>	3±3 <sup>a</sup>	3±3 <sup>a</sup>
	<i>S. indicus</i> female	2±3 <sup>a</sup>	3±3 <sup>a</sup>	3±3 <sup>a</sup>
	All species	81±70 <sup>a</sup>	38±27 <sup>a</sup>	58±40 <sup>a</sup>

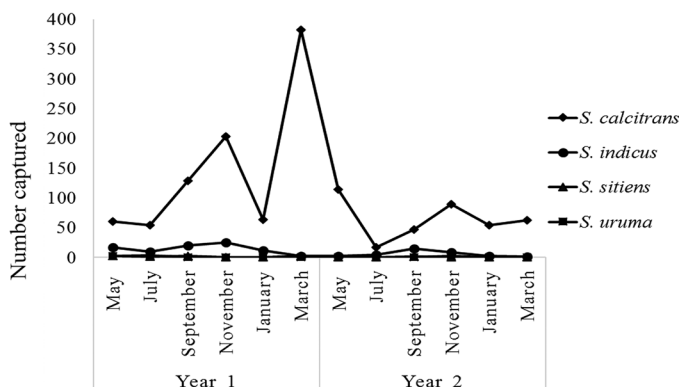
Mean ± SD in the same row superscripted with different lowercase letters indicate a significant ( $p < 0.05$ ) difference between seasons.



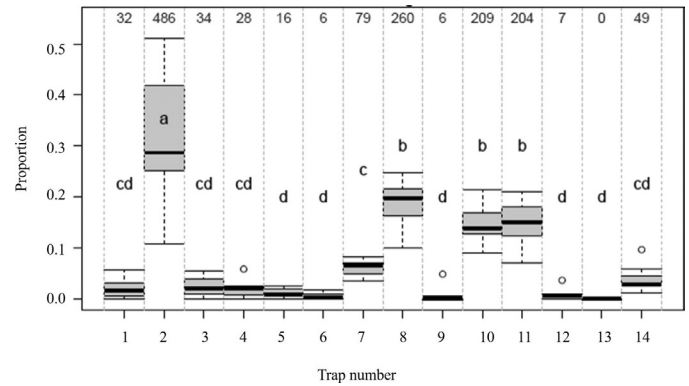
**Fig. 1** Numbers of *Stomoxys* spp. trapped per month on a beef cattle farm



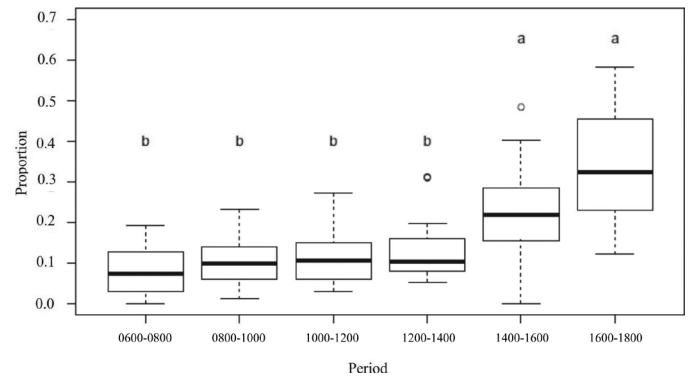
**Fig. 2** Boxplots of mean stable fly trap count using 10 Vavoua traps on a beef cattle farm where different lowercase letters denote significant different ( $p < 0.05$ ); figures at the top of each trap indicate total number of flies caught; upper and lower horizontal lines in a box represent the 25<sup>th</sup> and 75<sup>th</sup> percentiles, respectively; horizontal midlines represent median; upper and lower horizontal whiskers lines are upper and lower limits and open circles are outliers.



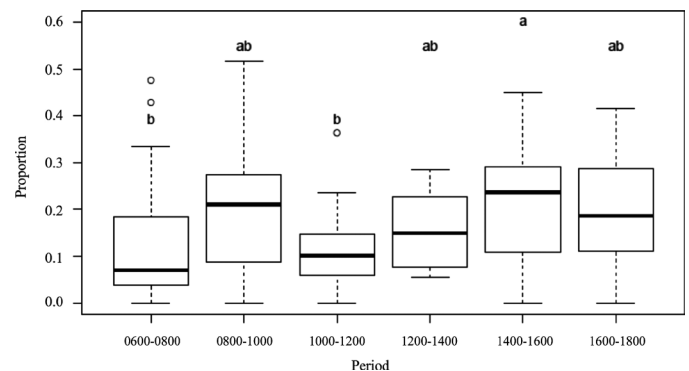
**Fig. 3** Captures of *Stomoxys* spp. per month at Khao Kheow Open Zoo



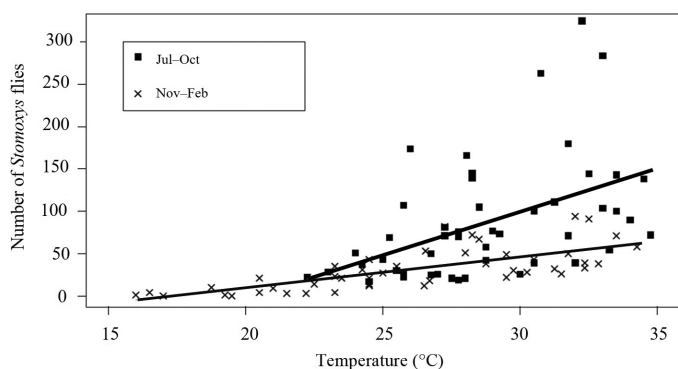
**Fig. 4** Boxplots of mean stable fly trap count using 14 Vavoua traps at Khao Kheow Open Zoo where different lowercase letters denote significant different ( $p < 0.05$ ); figures at the top of each trap indicate total number of flies caught; upper and lower horizontal lines in a box represent the 25<sup>th</sup> and 75<sup>th</sup> percentiles, respectively; horizontal midlines represent median; upper and lower horizontal whiskers lines are upper and lower limits and open circles are outliers.



**Fig. 5** Boxplots of proportions of stable flies trapped per 2 hr period using Vavoua traps on a beef cattle farm where different lowercase letters denote significant different ( $p < 0.05$ ); upper and lower horizontal lines in a box represent the 25<sup>th</sup> and 75<sup>th</sup> percentiles, respectively; horizontal midlines represent median; upper and lower horizontal whiskers lines are upper and lower limits and open circles are outliers.



**Fig. 6** Boxplots of proportions of stable flies trapped per 2 hr period using Vavoua traps at Khao Kheow Open Zoo where different lowercase letters denote significant different ( $p < 0.05$ ); upper and lower horizontal lines in a box represent the 25<sup>th</sup> and 75<sup>th</sup> percentiles, respectively; horizontal midlines represent median; upper and lower horizontal whiskers lines are upper and lower limits and open circles are outliers.



**Fig. 7** Correlation between number of *Stomoxys* flies and temperature in rainy (Jul–Oct) and cool (Nov–Feb) seasons on a beef cattle farm, where thick line is fitted data (predicted data shown as filled squares) for the rainy season and thin line is fitted data for the cool season (observed data shown as crosses)

Research on Stomoxys flies has mostly taken place in the USA, Europe and Africa (Mavoungou et al., 2008; Dsouli et al., 2011; Dsouli-Aymes et al., 2011; Taylor et al., 2012; Ose and Hogsette, 2014; Machtinger et al., 2016), but in recent years there has been research output from Thailand (Malaithong et al., 2019), with most of the latter work reporting on species identification and diversity, seasonal and diurnal activity and control methods. Six species of Stomoxys flies are known from Thailand, among which the most widely distributed in rural and semi-urban settings is *S. calcitrans* (Masmeatathip et al., 2006; Muenworn et al., 2010; Changbunjong et al., 2012; Phasuk et al. 2016; Malaithong et al., 2019).

The present survey compared two completely different habitats—a small beef cattle farm (BCF) and a zoo (KKOZ)—over a 2 yr period, with *S. calcitrans* being dominant in both locations. Out of the 10,174 specimens collected altogether, *S. calcitrans* was the most abundant species, followed by *S. indicus*. Most of the flies (86.1%) were trapped on BCF and only 13.9% at KKOZ. These findings agreed with other studies that reported extremely high numbers of *S. calcitrans* on dairy farms compared with forest settings, indicating that *S. calcitrans* is clearly associated with anthropic ecological settings (Muenworn et al., 2010; Changbunjong et al., 2012; Phasuk et al., 2016). *S. sitiens* and *S. uruma* were trapped in very low numbers in both habitats surveyed, whereas Changbunjong et al. (2012) reported large numbers of *S. uruma* collected in Khao Yai National Park. This species, which has long been known from other Asian countries such as Hong Kong, India, Vietnam and Taiwan (Zumpt, 1973), was only recently reported from Thailand by Muenworn et al. (2010). In contrast to *S. calcitrans*, *S. uruma* could be associated with areas with a higher degree of naturalness.

The lower number of Stomoxys flies collected at the zoo could have been related to the cleanliness of the zoo restricting potential breeding sites for flies. KKOZ is a public organization and is committed to clearing away most animal wastes, which are potential breeding habitat for most Stomoxys flies and other insects and pests, and to composting them to produce a valuable resource, while ensuring a high level of hygiene on the premises. As a result, the zoo's environment may have lacked sufficient suitable media for fly development, despite having a higher number of Bovidae than on the farm. In a different situation, Mihok and Clausen (1996) collected Stomoxys flies using Vavoua traps in a forested area in Kenya and caught flies at very low densities.

Other studies have shown that fly abundance was related to environmental factors such as temperature, relative humidity, rainfall, and light intensity to sustain favorable conditions in breeding habitats, as well as to biotic factors such as the distribution and abundance of animal hosts (Masmeatathip et al., 2006; Changbunjong et al., 2012; Keawrayup et al., 2012). Masmeatathip et al. (2006) reported that the peak density of *S. calcitrans* coincided with rainfall, whereas Muenworn et al. (2010) observed a major peak in *S. calcitrans* abundance during the hot season. In the current study, *S. calcitrans* and *S. indicus* were most abundant during the rainy season on BCF. In this environment, the abundance levels of both species peaked in August in both years of the survey. In contrast, no clear, dynamic, yearly pattern of abundance could be noted over the 2 yr of the survey at KKOZ, probably due to repeated disturbances linked to the management of animals and the frequent dung removal.

Similar differences were observed regarding the diurnal activity of flies. On BCF, more flies were trapped in the afternoon (1400–1800 hours). The herd of cattle was managed the same way every day throughout the year. At KKOZ, no peak of daily activity was noted, probably linked with the general management of the area and the wide range of animal species present on the site, each with distinctive behavioral characteristics.

Muenworn et al. (2010) observed a peak in the daily flight activity for *S. calcitrans* during 1000–1600 hours. Masmeatathip et al. (2006) reported bimodal activity of *S. calcitrans*, with a first peak during 0800–1000 hours and a second, less marked one during 1600–1800 hours. Elsewhere, several studies have reported bimodal activity of *S. calcitrans* in Florida, USA (Simmonds, 1944), Mauritius (Kunz and Monty, 1976), Manaus, Brazil (Charlwood and Lopes, 1980) and Kansas, USA (Semakula et al., 1989), whereas a unimodal activity pattern of *S. calcitrans* was observed in Uganda (Coaker and Passmore, 1958; Harley, 1965).

In conclusion, the present study provided information on Stomoxyine flies found at a small beef cattle farm and a zoo, with 10,174 individuals of four fly species being trapped. The results confirmed that *S. calcitrans* and *S. indicus* are the predominant species in these anthropic ecological settings, but their numbers were considerably higher on the farm (only 20 head of cattle), than at the zoo, where more than 3,000 animals were present. Differences in the management of the zoo animals and their waste could explain this discrepancy. Further research is required to understand the factors underpinning the surprisingly low Stomoxyine fly activity observed at the zoo.

### Conflict of Interest

The authors declare that there are no conflicts of interest.

### Acknowledgements

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