TEMPORAL AND SPATIAL DISTRIBUTION, SEX RATIO AND FECUNDITY OF THE EYE FLY *SIPHUNCULINA FUNICOLA* (DIPTERA: CHLOROPIDAE) AT AGGREGATION SITES DURING DIURNAL AND NOCTURNAL PERIODS

Uruyakorn Chansang[1], Mir S Mulla[2] and Pathom Sawanpanyalert[3]

[1]Taxonomy and Museum Reference Section, National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand; [2]Department of Entomology, University of California, Riverside, California, USA; [3]National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand

Abstract. The present study was aimed to determine the distribution and abundance of the eye fly *Siphunculina funicola* (de Meijere) in Thailand and to investigate the sex ratio and fecundity of eye flies from aggregation sites collected during the day-time and night-time. The flies were collected from several provinces in central Thailand and Phuket in the south. Observations were regarding the relative abundance of eye flies in different regions and seasons. During 2007 and 2008, large populations of eye flies were noted at resting sites in central Thailand with both day and night collections. Males flies outnumbered female flies. Smaller populations were seen in Chumphon and Surat Thani Provinces with increasing numbers in Krabi and Phuket Provinces in the south. The gravid rate was nil in the few females collected in Chomphon and Surat Thani but were 3.9% and 36.3% in Krabi and Phuket, respectively. The gravid rates were higher during the dry season or during dry spells than during wet and rainy periods, suggesting egg retention by the females when oviposition sites (presumably soil) were dry. Numerous day and night collections were made in Chon Buri Province. In most collections males predominated but there was no differences in the numbers of flies collected during the two time periods. There was a slightly greater percentage of females (still lower than males) during the night collections. During the dry and hot season, due to lack of optimum oviposition sites because of dryness, the eggs were retained in the females. A series of day time collections at the end of April 2008 and in February-March 2009 had higher numbers of gravid females. Day time collections in May 2008 (start of the rainy season) showed a moderate number of gravid females, but the gravid rates were low during the rainy season, indicating higher oviposition activity by females.

Keywords: *Siphunculina funicola*, aggregation, sex ratio, fecundity, Thailand

Correspondence: Dr Uruyakorn Chansang, National Institute of Health, Department Medical Sciences, Ministry of Public Health, Nonthaburi 11000, Thailand.
Tel: 66 (0) 2951 0000 ext 99243; Fax: 66 (0) 2591 5449
E-mail: uruyakorn.c@dmsc.mail.go.th

INTRODUCTION

The eye fly *Siphunculina funicola* (de Meijere) commonly known as the oriental eye fly is a severe human and animal pest and a potential vector of pathogenic organisms to humans and domestic ani-

mals. It is widely distributed in South and Southeast Asia. It has been implicated in carrying and transmitting the causal agent of catarrhal opthalmia (Graham Smith, 1930; Syddiq, 1938; Muirhead-Thomson, 1954). Eye flies are a neglected group of human pests and potential disease vectors, with few studies reporting their bioecology and behavior to provide a basis for the practical and effective management of eye flies in agricultural, residential and recreational areas. From the standpoint of their ubiquitous presence in infested areas and the persistent hovering and feeding activity of both male and female flies, they constitute an important and widespread group of potential disease vectors. In addition to their potential as vectors for pathogenic organisms, eye flies land and attempt to feed on sweat, wounds, scratches, lacerations, moist skin, eyes, lips and other moist and oozing areas of humans and animals (Graham-Smith, 1930; Mulla and Chansang, 2007; Chansang and Mulla, 2008a,b).

Mulla and Chansang (2007) showed for the first time that both male and female eye flies are anthropophilic and zoophilic and feed on moist surfaces, wounds, scratches and other parts of the host body. These authors also found that a substantial number of eye flies (both males and females) form aggregations, mostly on vertically hanging structures, such as ropes, sheaths, wires, strands, threads, bird nest trailings, coconut or onion hairs, cob webs, hangings from eaves, beams and door jams and ceilings of structures, such as decks, porches and thatch roofs. Mulla and Chansang (2007) developed procedures to quantitatively sample eye flies in these aggregations to study their biology and behavior. During the course of these studies they noted the aggregations were present during both the day and night. The flies at night were not active, only being able to fly or flutter around on the resting substrate. We postulated some of the flies might leave the aggregations before sunset. The females are thought to fly to breeding grounds which we believe are agricultural fields and other moist soil and landscaped areas. We also attempted to determine if gravidity rates decline at night. Another important parameter studied was female gravidity rates in different geographical areas and at different times. Aggregations were collected, the flies' sex and gravidity rates determined. Collections of aggregations from Bang Lamung District (Chon Buri Province) and from 3 districts in Chachoengsao were made and analyzed microscopically for the objective parameters.

MATERIALS AND METHODS

Study sites and collections

Most of our studies were carried out in two central provinces (Chon Buri and Chachoengsao) and limited observations were made in southern Thailand (Chumphon, Surat Thani, Krabi and Phuket Provinces). All day time collections were made from eye fly aggregation sites as shown in Fig 1 in Bang Lamung District (Chon Buri Province) and Chachoengsao Province during August 28-30, 2007. Two collections were made by sweeping hovering eye flies from around human hosts in village 2 (Ban Mab Charoen) in Bang Lamung District (Chon Buri Province). Day time collections were also made from resting sites in Chumphon, Surat Thani, Krabi and Phuket during January 21-27, 2008, with one collection from humans in Chumphon. During August 19-20, 2007, February 7-8 and 11-13, 2008 and April 29-May 2, 2008, several day and night time collections (see details

(a) Broom sticks in a car port.

Fig 1–Examples of aggregation sites of eye flies where the flies were collected for determination of sex ratio and fecundity, (a) some sticks of a broom placed under the roof of a car port; (b) nylon rope hanging from the ceiling of a house.

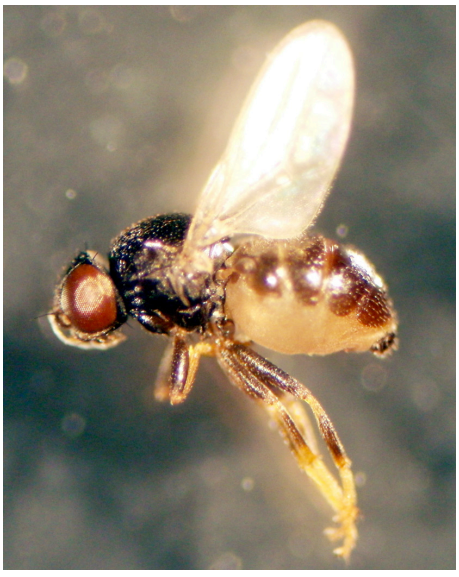
below) were made in Chon Buri and Chachoengsao Provinces to determine sex ratio and fecundity.

Collection methods

Collections from aggregation sites were made using an insect killing jar method (Mulla and Chansang, 2007). An insect killing jar was pushed up vertically against the hanging aggregation. When the substrate with the eye flies was completely enclosed in the jar, the aggregation was shaken. This process dislodged the resting eye flies from the substrate causing them to fall into the insect killing jar, then the jar was capped. The eye flies in the jar were instantly killed, then transferred to bottles with 70% ethanol to preserve the flies, labeled and then transported to the laboratory. The eye flies collected by sweep nets around humans were transferred to insect killing jars, killed and then preserved in 70% ethanol. Flies from wounds were also collected as they flew from the wound. Some eye flies at resting sites were collected in 70% ethanol, by pushing up a plastic bottle half full of ethanol enclosing the flies, then shaking the flies into the alcohol.

All collected specimens were brought to the laboratory counted and sexed under a stereomicroscope. The male eyefly is characterized by the claspers at the tip of the abdomen which may be extruded or retracted (Fig 2a,b), while the female is recognized by the ovipositor caudal to the last abdominal segment. The ovipositor may be extruded or retracted (Fig 2c,d). After counting and sexing, the females were dissected under a stereomicroscope for the presence of eggs as shown in Fig 3a. With mature eggs, it is not necessary to dissect the females as the eggs can be easily seen through the venter of the abdomen under a stereomicroscope (Fig 3b).

(a) Male extruded claspers.



(b) Male retracted claspers.

(c) Female extended abdomen and ovipositor.

(d) Female retracted ovipositor.

Fig 2–Male and female terminalia of *Siphunculina funicola.* Male: (a) with extruded claspers, (b) with retracted claspers, and female: (c) extended abdomen and ovipositor, (d) retracted abdomen and ovipositor.

To count the number of mature eggs, the female flies were dissected by pulling the tip of the abdomen open with fine forceps and exposing the mature eggs which resemble rice grains (Fig 3a).

Day and night collections

Since both males and females are present in the aggregations (Mulla and Chansang, 2007), we postulated the females might leave for oviposition sites

(a) Eggs dissected out, which resemble rice grains.

(b) Eggs visible through the venter of the abdomen.

Fig 3–Mature eggs in a gravid eye fly *Siphunculina funicola,* (a) dissected out, which resemble rice grains, and (b) eggs visible through the venter of the abdomen.

before the dark, inactive period. To evaluate this hypothesis we determine the sex ratio and fecundity of the eye flies removed from their aggregation sites during the day and night. The daytime collections were made 1-3 hours before and the night time collections 2-3 hours after sunset. Eye flies are quite active during the day at the resting sites but this activity ceases soon after sunset (Mulla and Chansang, 2007). The samples were collected and processed in the same manner as described above and taken to the laboratory for counting, sexing and to determine the presence and number of eggs in the females.

Statistical analysis

A paired *t*-test (Statistix, 2003) was used to compare the day and night collection sex ratios (see Tables).

RESULTS

Two day time collections from several aggregations were made in Chon Buri Province during the rainy season (August 28-30, 2007). The results of these collections are presented in Table 1. There were collections of 7 and 8 aggregations taken from village 2 (Ban Mab Charoen) and village 4 (Ban Kai Nao), respectively. In all aggregations males outnumbered females, the overall mean proportions of females from the two villages were 33.2% and 30.6%, respectively. The gravidity rates in females ranged from 0.8% to 0.9%; the expected rates are quite low during the rainy season (rainfall in August usually 145-150 mm).

To further explore the abundance, sex ratio and fecundity of aggregated populations, collections were made during a relatively dry season in February, and in much drier conditions in March 2009 in Chon Buri and Rayong Provinces to see if weather conditions influenced those parameters. The abundance of eye flies in February (still wet) was relatively low in the two village collections at the end of the wet season (total rainfall in February was 45 mm), but the gravidity rates were

Table 1
Abundance, sex ratio and fecundity of the eye fly *Siphunculina funicola* collected from aggregation sites during the day time from villages in Chon Buri and Rayong Provinces, Thailand (August, 2007, February and March, 2009).

Province (district/village)	Aggregation site no. and type		Eye fly numbers		Gravid females (%)[a]
			Males	Females	
Chon Buri			28-30 August 2007		
(Bang Lamung/	1 Plastic rope		111	55	1.8
Ban Mab Charoen)	2 Plastic rope		330	130	0
	3 Thatch roof		225	107	0
	4 Thatch roof		75	44	0
	5 Bird nest trailing		604	286	0.7
	6 Bird nest trailing		365	135	1.5
	7 Nylon rope		898	538	2.1
Means significantly different at $p < 0.01$[b]		Mean	373	185	0.9
		%	66.8	33.2	-
Chon Buri			28-30 August 2007		
(Bang Lamung/	8 Thatch roof		246	102	1.0
Ban Kai Nao)	9 Thatch roof		71	173	0.6
	10 Thatch roof		138	27	3.7
	11 Corn husk		141	16	0
	12 Electric line		756	253	0.8
	13 Electric wire		212	87	0
	14 Cob web		77	32	0
	15 Rope strand		338	181	0.6
Means significantly different at $p = 0.05$[b]		Mean	247	109	0.8
		%	69.4	30.6	-
Chon Buri			22-23 February 2009		
(Bang Lamung/	1 Broom strand		56	41	29.3
Ban Mab Charoen-	2 Broom strand		127	96	18.8
Chicken farm)	3 Broom strand		131	114	13.2
	4 Broom strand		130	49	18.4
Means significantly different at $p = 0.10$[b]		Mean	111	75	19.9
		%	59.7	40.3	-
Chon Buri			22-23 February 2009		
(Bang Lamung/	1 Rope strand		42	36	22.2
Ban Kai Nao)	2 Plastic strand		123	76	22.4
	3 Thatch roof		30	55	2
Means not significantly different at $p < 0.69$[b]		Mean	82	56	22.3
		%	59.6	40.4	-
Chon Buri			15-16 March 2009		
(Bang Lamung/	1 Nylon rope		210	155	30.3
Ban Mab Charoen)	2 Broom strand		136	153	20.3
	3 Broom strand		310	314	17.8
Means not significantly different at $p = 0.66$[b]		Mean	218.7	207.3	22.8
		%	51.3	48.7	-

Table 1 (Continued).

Province (district/village)	Aggregation site no. and type		Eye fly numbers Males	Eye fly numbers Females	Gravid females (%)[a]
Chon Buri			15-16 March 2009		
(Bang Lamung/ Ban Kai Nao)	1 Nylon rope		137	110	26.4
	2 Wood strand		312	345	17.4
	3 Plastic strand		109	87	35.6
	4 Rubber stripe		993	1,062	17.0
	5 Nylon rope		158	181	24.9
	6 Cloth decoration		651	774	20.2
	7 Dead leaf and Cob web		142	130	17.0
Means not significantly different at $p < 0.24$[b]		Mean	357.4	384.1	22.6
		%	48.2	51.8	-
Rayong			15-16 March 2009		
(Ban Chang/ Ban Sam Nak Thon)	1 Plastic strand		303	354	17.8
(Ban Chang/ Ban Chakmark)	2 Wood strand		306	387	16.3
(Nikhom Phatthana/ Ban Kaset)	3 Rope strand		118	77	2.6
(Nikhom Phatthana/ Ban Tetsaban)	4 Rope strand		402	375	8.0
(Mueang/ Ban Kao Boat)	5 Nylon rope		98	71	1.4
Means not significantly different at $p < 0.78$[b]		Mean	245.4	252.8	9.2
		%	49.3	50.7	-

[a]All females in samples inspected for presence of mature eggs.
[b]Paired *t*-test

higher (20-22%) in this drier period compared to August (total rainfall in August >150 mm) collections (0.8-0.9%) (Table 1). The proportion of females was higher in February, indicating reduced oviposition in drier habitats when females prefer to stay in protected resting sites. Collections in March (total rainfall <30 mm) in Bang Lamung District, showed a relatively high abundance of eye flies on resting sites (Table 1), but confirmed higher gravidity rates in the dry than the wet season (Table 1) as well as a higher proportion of females, supporting our supposition that gravidity rates due to egg retention are higher in the dry than the wet season, and the higher number of females in the dry season is due to lower oviposition activity in the dry season.

During March (total rainfall 45 mm) collections were made in Rayong Province. The abundances of eye flies at resting sites were higher in Bang Lamung District in Chon Buri Province. We attribute this fact to increased moisture, since the plants looked healthier than the plants in Bang Lamung which were on the verge of wilting. In the 5 collections from 3 districts in Rayong, females constituted 50.7% of the population (Table 1). The average gravidity

Table 2
Abundance, sex ratio and fecundity of the eye fly *Siphunculina funicola* collected from aggregation sites or swept from humans during the day in southern Thailand (January, 2008.)

Province (district)	Aggregation site or off human	Eye fly numbers		Females dissected	Gravid females (%)
		Males	Females		
Chumphon (Mueang)	Off human	6	4	4	0
	Wood strand	38	17	17	0
Surat Thani (Punpin)	Plastic rope	14	9	9	0
Krabi (Ao Phra Nang)	Strand	284	254	254	3.9
Phuket (Talang)	Electric cord	3,557	3,159	200[a]	36.3

[a]Because of the large number of flies, only 200 females selected at random were inspected for presence of mature eggs. Mean number of eggs per female was 47.5.

rate was lower than those from Bang Lamung District.

A few small collections of eye flies were made during the day time in the dry season (January) in southern Thailand. In the 4 collections, one each from Chumphon, Surat Thani, Krabi and Phuket Provinces, the proportion of females was lower than males. The females had lower gravidity rates except in Phuket where the gravid rate was 36.3% (Table 2). Low numbers of eye flies were collected in Chumphon and Surat Thani Provinces. However, in Krabi and Phuket, the resting sites were heavily loaded, especially at one site (electric cord) in Talang District, Phuket which was loaded with over 20,000 eye flies, from which 6,716 eye flies were collected for analysis (Table 2). In Chumphon and Surat Thani few flies were collected from resting sites, and at these sites, females were fewer than males, and none were found to be gravid. Large numbers of eye flies were collected in Krabi and Phuket where females constituted 47% of the flies at both locations. The gravidity rate in Krabi (more rain in January) was 3.9%, while in Phuket (dry location) it was 36.3%, further supporting our view of egg retention by females under dry conditions.

Sex ratio and gravidity rates in diurnal and nocturnal collections

After making several daytime collections to determine sex ratio and gravidity, we then focused our attention on comparing sex ratios and gravidity rates diurnally and nocturnally in Ban Mab Charoen Village and Ban Kai Nao Village of Bang Lamung District, Chon Buri Province during the rainy season of August 2007 (total rainfall in August 80.2 mm). At each village 7 diurnal and 7 nocturnal collections were made. The substrates at each site were matched, one being collected during the day and the other during the night. All the collections (28), with the exception of two nocturnal collections, contained lower proportions of female eye flies (Table 3). In two nocturnal collections from Ban Mab Charoen Village, the ratio of females was higher than males (Table 3). The mean overall percentages of females in Ban Mab Charoen Village were 41.8% and 46.8% for the diurnal and nocturnal collections, respectively. In both the diurnal and nocturnal collections, the

Table 3
Abundance, sex ratio and fecundity of the eye fly *Siphunculina funicola* collected from their aggregation sites during the day and night time periods, in Bang Lamung District, Chon Buri Province, Thailand (August, 2007).

Village and no.	Day time collections[a]				Night time collections[a]			
	Site and type	Eye fly numbers		Gravid (%)	Site and type	Eye fly numbers		Gravid (%)
		Males	Females			Males	Females	
Ban Mab Charoen (Village 2)	AA Thatch roof	113	49	2.0	BB Thatch roof	366	415	0
	CC Thatch roof	79	74	0	DD Thatch roof	771	498	0
	EE Electric line	149	70	1.4	FF Wire	431	483	0.2
	GG Electric line	189	175	0	HH Wood strand	103	72	0
	II Nylon string	250	113	0.9	JJ Bamboo and wire	62	52	0
	KK Plastic strand	861	678	0.3	LL Coat hanger	395	359	0
	MM Thin wire	179	148	0.7	NN Bird nest trailing	235	200	0
Males day *vs* night not significant at $p<0.60$[b]	Mean	260	187	0.8	Females day *vs* night not significant at $p<0.36$[b]	338	297	0.03
	%	58.2	41.8	-		53.2	46.8	-
Ban Kai Nao (Village 4)	A Plastic string	159	95	0	B Plastic string	60	42	0
	C Plastic string	147	59	0	D Plastic string	328	223	0
	E Plastic string	165	99	0	F Plastic string	61	34	0
	G Plastic string	475	285	0	H Plastic string	162	109	0
	I Plastic string	178	152	0	J Plastic string	1,470	1,113	0
	K Thatch roof	245	254	0.4	L Plastic string	256	227	0.9
	M Thatch roof	180	183	0.5	N Plastic string	389	286	0
Males day *vs* night not significant at $p<0.43$[b]	Mean	221	161	0.13	Females day *vs* night not significant at $p<0.40$[b]	389	291	0.13
	%	58.0	42.0	-		57.3	42.7	-

[a]All females in the samples inspected for presence of mature eggs.
[b] Paired *t*-test

Table 4
Abundance, sex ratio and fecundity of the eye fly *Siphunculina funicola* in day and night time collections of flies from their aggregation sites in Chon Buri and Chachoengsao Provinces, Thailand, (February, 2008).

	Day time collections				Night time collections			
District and (village)	Site and type (length cm)	Eye fly numbers Males	Females	Gravid (%)[a]	Site and type (length cm)	Eye fly numbers Males	Females	Gravid (%)[a]
Chon Buri (February 11-13)								
Bang Lamung	1 Plastic rope (34)	370	329	0	2 Plastic rope (25)	664	593	0
(Ban Mab Charoen)	3 Bird nest trailing (54)	611	417	0	4 Plastic rope (30)	721	580	0
	5 Bamboo fish pole (26)	181	121	0	6 Flag (30x50)	538	473	0
	7 Electric, nylon cord (32)	335	241	0	8 Wood strand (15)	243	178	0
Males day *vs* night not significant at $p<0.20$[b]	Mean	374	277	0	Females day *vs* night not significant at $p<0.36$[b]	541	442	0
	%	57.5	42.5	-		55.0	44.9	-
Chachoengsao (February 7-8)								
Phanom Sarakham	1 Thread (15)	438	255	0	2 Thatch roof (15)	268	287	1
(Ban Nong Pradu Lai)	3 Thatch sheath (15)	262	159	0	4 Thread (15)	541	484	0
	5 Thatch sheath (25)	124	111	0	6 Thread (15)	170	161	0
Males day *vs* night not significant at $p<0.73$[b]	Mean	275	175	0	Females day *vs* night not significant at $p<0.36$[b]	326	311	0.3
	%	61.1	38.9	-		51.0	49.0	-

[a]All females in the samples inspected for presence of mature eggs.
[b]Paired *t*-test

Table 5
Abundance, sex ratio and fecundity of the eye fly *Siphunculina funicola* in day and night time collections of flies from their aggregation sites in 2 villages of Bang Lamung District, Chon Buri Province, Thailand, (April-May, 2008).

Village no.	Day time collections				Night time collections			
	Site and type (length cm)	Eye fly numbers		Gravid (%)[a]	Site and type (length cm)	Eye fly numbers		Gravid (%)[a]
		Males	Females			Males	Females	
Village 2	A Dry bean (20)	842	552	4.7	AA Plastic rope (20)	438	286	0.1
(Ban Mab Charoen)	B Wire (15)	421	169	2.4	BB Plastic rope (15)	236	131	5.3
	C Wood sheath (6)	115	61	1.6	CC Cord (15)	185	56	1.8
	D Wire (10)	25	16	0	DD Wire (16)	151	59	3.4
Males day *vs* night not significant at p=0.48[b]	Mean	351	200	2.2	Males day *vs* night not significant at p<0.40[b]	253	133	2.7
	%	63.7	36.3	-		66	34	-
Village 4	G Sack strand (8)	208	134	0	GG Plastic string (10)	6	7	0
(Ban Kai Nao)	H Thatch roof sheath (10)	82	98	10.1	HH Thatch roof sheath (15)	224	117	12.0
	I Thatch sheath (12)	572	638	7.2	II Thatch roof sheath (15)	150	49	10.2
	J Thatch roof sheath (10)	236	206	3.9	JJ Thatch roof sheath (12)	38	30	0
Males day *vs* night not significant at p<0.24[b]	Mean	275	269	5	Males day *vs* night not significant at p=0.19[b]	105	51	6
	%	50.6	49.4	-		67.3	32.7	-

[a]All females in the samples inspected for presence of mature eggs
[b]Paired *t*-test

Table 6
Abundance, sex ratio and fecundity of the eye fly *Siphunculina funicola* captured from human wounds, and off human hosts during the day at the community hall of Ban Mab Charoen (village 2), Bang Lamung District, Chon Buri Province, Thailand (April, 2008).

Source of collection[a]	Eye fly numbers		Gravid females (%)[b]
	Males	Females	
Wound 1	15	4	0
Wound 2	10	3	0
Wound 3	5	0	0
Wound 4	16	10	0
Wound 5	4	0	0
Off human	5	1	0
Mean	9.1[c]	3.0[c]	

[a]Eye flies from wounds collected as they flew away, not touching the wound.
[b]All females inspected for mature eggs; [c]Significantly different at $p = 0.002$

percents of gravid females were low at 0.8% and 0.03%, respectively, during the rainy season in August.

A similar picture for sex ratio and gravidity rates was seen for Ban Kai Nao Village during the rainy season. Of the 14 samples collected, 2 had lower or equal numbers of males than females. The overall female proportions were 42.0% and 42.7% for the diurnal and nocturnal collections, respectively. The gravidity rates were low, at 0.13% and 0.13% for the diurnal and nocturnal collections, respectively (Table 3).

In February 2008, a relativity dry month, diurnal and nocturnal aggregation collections were made in the villages of Ban Mab Charoen (Chon Buri Province) and Ban Nong Pradu Lai (Chachoengsao Province). In Ban Mab Charoen, there were 4 sites and in Ban Nong Pradu Lai there were 3 sites collected during the day and night. In both locations, there were many eye flies; the night collections had more eye flies than the day collections. In Ban Mab Charoen, male eye flies outnumbered females at all locations (Table 4), with mean male to female ratios of 57.5% to 42.5% during the day and 55.0% to 44.9% during the night (Table 4). The proportion of females was higher at night than during the day. The gravidity rate in the females was 0.0% during both day and night collections (Table 4), unlike that found in January (2008) and February-March (2009). January-February 2008, although the dry season received some rain (25 mm in February 2008).

In Chachoengsao in February 2008, in both the day and night collections, males outnumbered females at 5 sites, while the reverse was true in one night collection (Table 4). The overall mean sex ratio in the 3 day collections was 61.1% males and 38.9% females, while the sex ratio in the 3 night collections was 51% males and 49% females (Table 4). Similar to the August 2007 collections in Chon Buri Province, the mean proportion of females at night was higher than during the day. The gravidity

rate was 0.0% in 5 samples (day and night) and 1.0% in one sample (Table 4), rather low for the dry season. Although February is usually relatively dry, precipitation in February 2008 was substantial in Chachoengsao.

During the dry season in April-May 2008, diurnal and nocturnal aggregation collections were made in two villages in Chon Buri. In each village had 4 collection sites, both diurnal and nocturnal. The overall mean sex ratios (females to males) in village 2 were 36.3% and 63.7% for diurnal collections and 34% and 66% for nocturnal collections, respectively (Table 5). The proportion of females in the nocturnal collections was substantially lower than the diurnal collections (Table 5) for village 4. The gravidity at the beginning of the rainy season (total rainfalls in April 2008 and May 2008 were 82 and 70 mm, respectively) was low, ranging from 2.2% to 6% (Table 5), slightly higher in village 4 than in village 2.

In April 2008, during the start of the rainy season, eye flies were collected after flying from human wounds and off human hosts (without landing) in village 2 (Bang Lamung District). In all samples, males outnumbered females (Table 6). The numbers of eye flies collected from wounds and off humans were small, so it is difficult to make conclusions regarding sex ratios and gravidity rates, but there was an absence of gravidity in these flies.

DISCUSSION

At present time there are no sensitive, reproducible methods for sampling the adult eye fly *Siphunculina funicola*. The only methods available are fraught with sampling problems. Numbering host-seeking flies using the sweep net method cannot be fine tuned due to environmental factors, such as wind, light, temperature and variations in the attraction of individual hosts. Sampling populations at resting sites is more practical and provides a clearer measure of population abundance. This method also has inherent problems, such as type, size, length and prior use of resting substrates. Since different sizes, types and locations of substrates were sampled, the sizes and shapes of natural substrates were quite variable. This method introduces a number of variables that cannot be controlled. However, sampling of flies at resting sites provides the most practical and acceptable method for sampling adult eye flies. This method has been employed successfully in other studies of aggregation sizes and behavior and testing of insecticide sprays and aerosols against these pests (Mulla and Chansang, 2007; Chansang and Mulla, 2008). It should be noted that in nature adult *Siphunculina funicola* exist in two forms: as dispersed populations, such as in agricultural fields, landscaped areas, golf courses, and village landscapes and as aggregations, mostly on vertically hanging substrates in protected structures close to human and animal hosts (Mulla and Chansang, 2007). In our previous and current studies we employed collection of aggregated populations extensively and of host-seeking adults sparingly.

As for geographical distribution of *Siphunculina funicola* (during 2006-2008) heavy populations have been found in central Thailand, in Chon Buri, Chantaburi, Nakhon Ratchasima and Chachoengsao Provinces (Mulla and Chansang, 2007; Chansang and Mulla, 2008). We have added Sa Kaeo, Buri Ram, Khon Kaen, and Roi Et Provinces to this list where we observed and collected large numbers of eye flies in 2009. It is likely that populations occur in other provinces of Thailand.

In 2008, we found low small populations in Chumphon and Surat Thani Provinces, but relatively large populations in Krabi and Phuket Provinces in southern Thailand. Low to moderate numbers have been noted (without collections) by the authors in Loei, Maha Sarakham, Phitsanulok, Sukhothai, Phetchabun, Phrae, Nan, Payao and Chiang Rai, Nong Khai and other areas. It has been noted the abundance of eye flies is qualitatively correlated with edaphic (soil) factors. Higher numbers have been found in agricultural fields characterized by sandy, gravely or moist soil, where crops, such as pineapple, cassava and vegetables are grown. Small populations were detected in Chumphon and Surat Thani where oil palms are grown and the soil has heavy clay. We believe eye flies breed in sandy or gravelly to sandy loam soil with organic matters, receiving moisture from rain or irrigation. David (1960) was able to colonize this fly on sand and organic mixture, and Mulla (1962) found a closely related species, *Hippelates collusor,* in California, which propagated in sandy agricultural fields under cultivation and irrigation. *Siphunculina funicola* was previously described in Java (de Meijere, 1905) and we believe it is distributed all over Thailand and Southeast Asia. Heavy populations are found where suitable breeding grounds and environmental conditions are prevalent.

Looking at the bioecological characterizations of aggregate populations sampled by recently developed procedures (Mulla and Chansang, 2007), comparative data have been obtained regarding the relative abundance, sex ratio and fecundity of eye flies.

The number of eye flies varied per site by collection time. The sex ratio also varied by location and season. In all collections, females constituted 30.6% to 49% of the population. This variation is probably due to weather and farming practices. Precipitation regulates gravidity rates. During the rainy season, gravidity rates were very low or nil, increasing during the dry season (February to May). We postulate females retain eggs during the dry season when moist oviposition sites are scarce.

In summary, the density of eye flies at aggregation sites varies by type, size and location of resting sites and by weather conditions. Cool and windy conditions had smaller numbers of eye flies at resting sites. The abundance also varied by edaphic factors: sandy and gravelly soils with adequate moisture resulted in larger populations. Males were more prevalent than females in aggregation sites. This finding has a fitness advantage since females have to fly to adjacent breeding grounds for oviposition. The proportion of gravid females in practically all collections from aggregation sites in the rainy season was low, near zero, with the exception of a few collections, but was high during the dry season. The gravidity rate in host questing females was zero. This is to be expected as gravid females do not need to feed on hosts. Gravid females seek breeding grounds and oviposition sites. Gravid females were found in large numbers in aggregation sites in dry and very dry conditions when oviposition sites were scarce.

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