Exodus from a Breeding Place and the Time of Emergence from the Pupa of *Culex pipiens fatigans**

by BOTHA DE MEILLON,^a ANTHONY SEBASTIAN ^b and Z. H. KHAN,^c WHO Filariasis Research Unit, Rangoon, Burma

In the course of our studies on the behaviour of *Culex pipiens fatigans* under the natural conditions obtaining in Rangoon, Burma, we observed the time of exodus from a breeding place and the time of emergence from the pupa. Very little work of this nature appears to have been done on mosquitos in the field. Nielsen & Haeger ^d reported that there is a rhythm of emergence in *Aedes taeniorhynchus* and that the time of day at which peak emergence occurs is dependent on temperature. Most laboratory observations have been made on material brought in from the field. Haddow, Gillett & Corbet ^e found that *Aedes aegypti* exhibited no diurnal emergence rhythm or response to dark and light in laboratory experiments in Uganda.

It should be noted that our findings are strictly relevant to observations made in the field under existing conditions. We also performed laboratory experiments in which continuous observations were recorded on many hundreds of mosquitos without becoming aware of the very definite rhythms reported here.

Exodus of newly emerged C. p. fatigans from a breeding place

These observations were made in June during the monsoon rains, when the sky is mostly overcast and the light may become poor some time before the sun actually sets. It would be desirable to repeat them during the dry season, when clear skies prevail and the sunset is well defined.

Material and methods. The normal breeding places of C. p. fatigans in Rangoon in June are pit latrines, septic tanks and other places that are not readily flushed by heavy downpours. We therefore again selected the septic tank in the sheltered compound of the Bishop Bigandet Convalescent Home that was a prolific breeding site.^f The part of the tank used by us measured 28 ft \times 9 ft (8.5 m \times 2.75 m), the depth was 6 ft (1.8 m) and at the time of our observations the water was about 1 ft (0.3 m) from the top. There were two manholes that would normally effectively seal the tank off from either entering or leaving mosquitos, but two 6-in (0.15 m) air vents were open, the gauze having long ago rusted away, and through these the female mosquitos had entered and left without hindrance. These vents were closed during our observations. It is realized that this tank represents a special kind of breeding place and the behaviour patterns described here may not be so clearly defined or even discernible in the usual kind of habitat.

The trap was a simple wooden frame covered with wire gauze, and provided with a sleeve and a sloping three-ply roof to keep the rain out. The bottom was open and fitted over the manhole. A slide was provided at the base so that the mosquitos that had entered could be isolated, but this proved to be unnecessary and the catchers soon learned to leave the slide half or less open, since there was little tendency for mosquitos to re-enter the septic tank.

Two catchers provided with torches and aspirators were on duty for three hours at a time; each hour's catch was placed in a separate cage. The next morning the mosquitos were taken to the laboratory, killed, sexed and counted. Catching continued in this manner over 24 hours for seven days.

^{*} From the WHO Filariasis Research Unit, Rangoon, Burma. This investigation was supported in part by the United States Public Health Service Research Grant EF 00194.04 from the Division of Environmental Engineering and Food Protection to the World Health Organization.

^a Project Leader. Present address: South East Asia Mosquito Project, Smithsonian Institution, United States National Museum, Washington, D.C., USA.

^b Entomologist; seconded from the Directorate of Health, Burma.

^c Assistant Entomologist; seconded from the Directorate of Health, Burma.

^d Nielsen, T. & Haeger, J. S. (1954) Bull. ent. Res., 45, 757-768.

^e Haddow, A. J., Gillett, D. & Corbet, P. (1959) Ann. trop. Med. Parasit., 53, 123-131.

f See the paper on page 39 of this issue.

Hour ^a	Number	of mosquitos lea	wing tank		
	Males	Females	Total		
01.00	784	866	1 650		
02.00	749	720	1 469		
03.00	751	748	1 499		
04.00	539	421	960		
05.00	663	671	1 334		
06.00	598	564	1 162		
07.00	73	78	151		
08.00	86	90	176		
09.00	30	56	86		
10.00	38	25	63		
11.00	175	177	352		
12.00	53	75	128		
13.00	154	225	379		
14.00	196	232	428		
15.00	231	265	496		
16.00	300	366	666		
17.00	567	794	1 361		
18.00	1 009	1 218	2 227		
19.00	7 089	6 903	13 992		
20.00	4 463	5 315	9 778		
21.00	1 612	2 301	3 913		
22.00	1 958	1 701	3 659		
23.00	1 144	1 277	2 421		
24.00	1 102	1 233	2 335		
Total	24 364 (48 %)	26 321 (52 %)	50 685		

TABLE 1 HOURLY EXODUS OF C. P. FATIGANS FROM A SEPTIC TANK

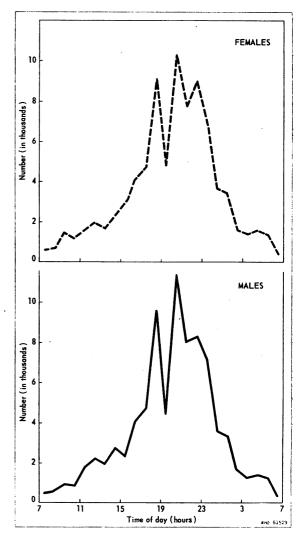
 $^{a}% \left(\mathbf{x}_{i}^{a}\right) =0$ The time stated is that at the end of the relevant hourly catch.

Results. The results of seven continuous catches from 8 June to 14 June 1965 are presented in Table 1. The catches for each 24 hours were so similar that they have been combined. It is clear that there is a marked peak for both males and females between 18.00 and 19.00 hours. It is during this time that sunset occurs.

Time of emergence of C. p. fatigans from the pupa

We have shown above that the clearly defined exodus of adults from a breeding place at about sunset applies to both males and females. This, how-

TIME OF EMERGENCE OF C. P. FATIGANS FROM PUPAE IN THE FIELD



ever, does not tell us anything about the time of emergence from the pupa, since it is conceivable that adults may emerge and then wait for some time before leaving the septic tank. It was therefore necessary to determine the exact time of emergence in a free-living colony in which the population was fairly stable and in which rafts were continually being deposited.

Material and methods. The breeding site was the septic tank described above. The large tray and shelter used in our oviposition studies g were em-

⁹ See the paper on page 39 of this issue.

TABLE 2. HOURLY EMERGENCE OF C. P. FATIGANS FROM PUPAE TAKEN FROM A SEPTIC TANK

Time ^a	14 July		15 July		16 July		17 July		20 July		21 July		22 July		23 July		24 July	
	ð	Ŷ	\$	Ŷ	ð	Ŷ	రే	ç	ð	Ŷ	3	Ŷ	3	ļ Ŷ	ð	ļ ç	ð	4
01.00			50	62	96	79	35	22			23	33	37	64	31	26	85	8
02.00			70	45	81	91	23	18			57	80	36	36	15	20	53	5
03.00			39	22	42	47	15	9			18	17	13	45	13	10	28	
04.00			43	29	13	21	4	8			8	11	33	42	10	11	20	1
05.00			25	18	22	31	11	5			18	13	31	52	17	22	19	1
06.00			23	18	8	10	14	9			4	20	30	27	18	22	31	2
07.00			8	3	2	5	11	11			5	3	0	5	2	2	9	
03.00			9	18	7	12	12	6			6	4	6	9	6	7	6	
09.00			17	27	7	11	10	13	3	4	9	7	2	4	4	2	3	
10.00			19	25	1	6	20	14	37	84	4	8	2	4	4	8	6	
11.00			12	19	1	5	12	7	41	63	8	9	1	6	6	5	4	
12.00	102	57	10	10	4	3			52	75	7	12	1	4	0	0		
13.00	109	49	7	15	3	2			91	118	5	11	1	4	5	3		
14.00	106	56	9	17	2	10			58	64	9	18	7	8	3	3		
15.00	138	78	2	13	4	4			102	101	14	19	11	9	3	4		}
16.00	48	31	21	39	16	7			117	181	19	22	8	7	3	8		
17.00	181	100	16	37	9	9			156	166	19	69	12	21	17	12		
18.00	230	125	17	26	16	21			117	185	39	56	28	47	21	16		
19.00	240	136	35	75	27	32			245	314	129	138	175	145	103	63		
20.00	95	72	17	30	9	12			95	144	36	57	135	94	61	37		
21.00	237	96	54	73	75	68			213	238	169	144	195	210	184	195		
22.00	163	108	110	171	70	70			97	96	139	132	81	88	145	105		
23.00	129	99	200	198	94	81			75	118	90	161	84	107	158	131		
24.00	116	79	214	166	54	51			58	86	62	123	89	81	129	117		

^a The time stated is that at the end of the relevant hourly catch.

ployed. The tray was filled each day with water from the septic tank containing larvae and pupae. No attempt was made to count the mosquitos in the immature stages but they were always present in excess.

Emerging adults were caught with a pair of forceps and immediately placed in alcohol. At first adults were picked up as soon as the pupal case ruptured. This, however, placed a heavy burden on the laboratory staff, who were generally faced with the laborious task of withdrawing the adult from the pupal case in order to determine its sex. We therefore instructed the collectors to pick up the adults when they were about halfway out of the case. At peak emerging times adults in all stages of emergence were collected.

The adults in alcohol were brought to the laboratory each day, counted and sexed. The collection of adults proceeded continuously over 24 hours for three days (14-16 July) and then again for four days (20-23 July).

Results. The data are presented in Table 2 and in the figure. It is immediately obvious that there is a definite peak of emergence between 20.00 and 21.00 hours for both sexes. In addition, the numbers of males and females that emerge are essentially equal at all times, the minor fluctuations observed not being

significant. Between sunrise and sunset 3379 males and 3421 females emerged and from sunset to sunrise, which includes the time of maximum emergence, 5066 males and 4999 females.

There is a marked decrease in the number emerging after sunset, between 19.00 and 20.00 hours. From Table 2 it will be seen that this occurred on every day of observation and that it applies to males as well as females. In spite of the closest examination we could not find any extrinsic factor, e.g., weather, laxity on the part of the collectors, that would account for this break in the rhythm.

TABLE 3. DEPENDENCE OF EXTENT OF ROTATION OF MALE TERMINALIA OF *C. P. FATIGANS* ON TIME ELAPSED SINCE EMERGENCE

Time since emergence (hours)	Extent of rotation of terminalia (amount of turn)					
1-2	None					
3	<1/4					
5-7	1/4					
7-8	1/2					
14	>1/2					
15-18	> 1/2; $3/4$					
19+	Full					

TABLE 4.	EXTENT OF ROTATION OF MALE T	ERMINALIA IN A SAMPLE OF
	C. P. FATIGANS ISSUING FROM A	SEPTIC TANK ª

Hour ^b	Number											
	examined	No turn		¹/₄ turn		¹/₂ turn		³/₄ turn		Full turn		
		No.	%	No.	%	No.	%	No.	%	No.	%	
01.00	50	36	72	8	16	3	6	0	0	3		
02.00	50	37	74	4	8	8	16	0	0	1	1	
03.00	50	36	72	1	2	5	10	0	0	8	1	
04.00	50	27	54	2	4	19	38	0	0	2	.	
05.00	50	28	56	o	0	4	8	1	2	17	3	
06.00	50	26	52	0	0	5	10	0	0	19	3	
07.00	50	16	32	4	8	26	52	2	4	2	.	
08.00	5	o	(0)	0	(0)	3	(60)	0	(0)	2	(4	
09.00	6	1	(17)	1	(17)	2	(33)	2	(33)	0	(
10.00	9	4	(44)	1	(11)	2	(22)	2	(22)	0	0	
11.00	35	9	(26)	1	(3)	10	(29)	0	(0)	15	(4	
12.00	50	6	12	0	0	10	20	3	6	31	6	
13.00	50	8	16	0	0	6	12	2	4	34	6	
14.00	50	11	22	0	0	7	14	3	6	29	5	
15.00	50	12	24	0	0	3	6	2	4	33	6	
16.00	50	6	12	1	2	8	16	1	2	34	6	
17.00	50	11	22	2	4	6	12	4	8	27	5	
18.00	50	16	32	0	0	2	4	2	4	30	6	
19.00	50	28	56	0	0	2	4	0	0	20	4	
20.00	50	31	62	1	2	7	14	0	0	11	2	
21.00	50	37	74	2	4	1.	2	0	0	10	2	
22.00	50	43	86	2	4	4	8	0	0	1	•	
23.00	50	42	84	2	4	4	8	0	0	2		
24.00	50	43	86	3	6	4	8	0	0	0		

 a Percentages in parentheses are not reliable since the numbers on which they are based are too small.

^b The time stated is that at the end of the relevant hourly catch.

Statistical analysis of the data obtained shows that the number collected between 19.00 and 20.00 hours is significantly different from that collected between 18.00 and 19.00 hours or between 20.00 and 21.00 hours. This is true for both sexes.

It is difficult to imagine what could cause this peculiar evening pause in the rhythm. We have observed previously that at about sunset some adult activities may be interrupted. This has been especially noticeable in biting catches and sugar-feeding cycles. Haeger h reported a similar finding in the nectar feeding of *Aedes taeniorhynchus* in Florida. He suggested that the break is probably due to the fact that it is at this time that observable swarming takes place. Our observations on *C. p. fatigans* swarming are too meagre to warrant the firm conclusion that swarming may also be responsible for the breaks in blood and sugar feeding observed by us. In any case, swarming certainly could not affect the rhythm of emergence.

As mentioned earlier, mass exodus from this particular septic tank takes place between 18.00 and 19.00 hours, coinciding with the first peak of emergence. The adults that make up the second peak of emergence apparently remain inside the tank until the next exodus at 19.00 hours the following night. This means that the 19.00-hours peak of exodus should be composed mostly of newly emerged adults and adults just under 24 hours old. This hypothesis was tested as follows.

The amount of rotation of the male terminalia can be used to determine age to within a few hours up to about the age of 20 hours, when rotation is complete. We know of no similar method for determining the age of young females, and our conclusions are therefore based on males only.

First we determined the rate of rotation in laboratory-bred males of known age; the data are presented in Table 3. We then examined up to 50 males from each of the hourly catches made over the septic tank. The results are shown in Table 4. From the percentages in each category and the known total catch for each hour, the total numbers in each category were calculated; the results are shown in Table 5. In this table the numbers in the quarter-, half-, and threequarter-turn categories are small, and comment is therefore restricted to the no-turn and full-turn categories.

It is clear that both these categories have a pronounced peak at about 19.00 hours, and this coincides with the peak for exodus. In other words, TABLE 5. ESTIMATED TOTAL NUMBER OF MALES OF *C. P. FATIGANS* IN SEVEN-DAY CATCH SHOWING SPECIFIED AMOUNT OF ROTATION OF THE TERMINALIA ^{*a*}

Hour ^b	Number		Estimated no. of males with terminalia showing rotation of							
Hour	leaving	No turn	1/4 turn	1/2 turn	³/₄ turn	Full turn				
01.00	784	564	125	49	0	47				
02.00	749	554	60	120	0	15				
03.00	751	541	15	75	0	120				
04.00	539	291	22	205	0	22				
05.00	663	371	0	53	13	225				
06.00	598	311	0	60	0	227				
07.00	73	23	6	38	3	3				
08.00	86	0	0	52	0	34				
09.00	30	5	5	10	10	0				
10.00	38	17	4	8	8	0				
11.00	175	45	5	50	0	75				
12.00	53	6	0	11	3	33				
13.00	154	25	0	18	6	105				
14.00	196	43	0	27	12	114				
15.00	231	55	0	14	9	152				
16.00	300	36	6	48	6	204				
17.00	567	125	23	68	45	306				
18.00	1 009	323	0	40	40	605				
19.00	7 089	3 970	o	284	0	2 836				
20.00	4 463	2 767	89	625	0	982				
21.00	1 612	1 193	64	32	0	322				
22.00	1 958	1 684	78	157	0	39				
23.00	1 144	961	46	92	0	46				
24.00	1 102	948	66	88	0	0				
Total	24 364	14 858	614	2 224	155	6 512				

^a The numbers are calculated on the basis of the percentage composition of each hourly catch computed in Table 4.

^b The time stated is that at the end of each hourly catch.

the peak for exodus is largely (80%) composed of mosquitos from these two classes—namely:

(1) newly emerged adults that have a peak at 19.00 hours and that belong to the "no-turn" class and

(2) older adults, which show a peak at 21.00 hours and which, having missed the 19.00-hour exodus, wait at the breeding place until the next night, when they leave together with newly emerged adults. They largely belong to the "full-turn" category and are therefore at least 19-20 hours old (Table 3).

^h Haeger, J. S. (1955) Mosquito News, 15, 21-26.