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The Duration of Egg, Larval and Pupal Stages of *Culex pipiens fatigans* in Rangoon, Burma*

BOTHA DE MEILLON,[1] ANTHONY SEBASTIAN[2] & Z. H. KHAN[3]

Laboratory experiments to determine the duration of the immature stages of Culex pipiens fatigans *were carried out because such information is important from the point of view of control by larvicides. At a temperature of 28.1°C±0.7°C the mean incubation period is 27.11±0.57 hours. Females spend a longer time in the pupal stage than males (34.16±0.74 hours and 32.95±0.75 hours, respectively, at 28.6°C±0.8°C; there is no 24-hour pupating or emerging rhythm. The duration of larval life is longer for the female (135.3±4.4 hours) than for the male (118.4±2.4 hours). Larvae that take a long time to pupate also take a long time to emerge. Withholding of food for a few hours from first-stage larvae increases the duration of larval life but does not affect that of pupal life. These observations on the differences between the sexes in the duration of larval and pupal life are in agreement with observations made on* Aedes aegypti *in Uganda.*

This investigation was undertaken during work on the laboratory biology of *Culex pipiens fatigans*. It has some practical application in local mosquito control since the length of time spent in the immature stages has a bearing on the spacing of insecticide spraying against larvae. It is well known that temperature changes have a marked effect on the duration of the immature stages, but this is not of great importance in Rangoon, where the mean temperature does not change much throughout the year (Table 1).

TABLE 1
MEAN MONTHLY TEMPERATURES IN RANGOON, 1951-60

Month	Mean temp.	
	°F	°C
January	79.3	26.3
February	80.9	27.2
March	84.0	28.9
April	86.9	30.5
May	85.3	29.6
June	81.9	27.7
July	81.4	27.4
August	81.2	27.3
September	81.8	27.7
October	82.9	28.3
November	82.4	28.0
December	79.1	26.2
Annual	82.3	27.9

THE INCUBATION AND HATCHING PERIODS OF *C. P. FATIGANS* EGGS

For convenience two terms will be used in this study: (1) *incubation period*, which is the time from the deposition of the raft to the appearance of the fully developed embryo, heralded by the appearance of the first free-swimming larva; and (2) *hatching period*, the time taken for all the viable larvae in the raft to hatch.

It is generally realized that, within certain limits, the incubation period of the egg is determined by the temperature. Recently, Shriver & Bickley (1964) reported that, in experiments in which the temperature was carefully controlled, the optimum temperature range for embryonic development was 75°F-85°F (23.9°C-29.4°C); under these conditions about 70% of the eggs hatched in about 30 hours. They

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

[2] Entomologist; seconded from the Directorate of Health, Burma.

[3] Assistant Entomologist; seconded from the Directorate of Health, Burma.

worked with a colony that originally came from Malaya and that had probably been cultured in the laboratory for some time, so that selection had undoubtedly taken place. Shriver & Bickley referred to similar work carried out by Kirkpatrick (1925) and de Boissezon (1930), in Egypt and France respectively, but it is not known whether they were working with *C. p. fatigans*.

Material and methods

The adults were reared from specimens obtained in the suburbs of Rangoon. They were fed on chicken and the resulting gravids were used for oviposition experiments. It was established that the rafts obtained in these experiments were complete; a number of adults dissected after oviposition had no retained eggs. The adults were under constant observation and the times when the act of oviposition started or finished are accurately known. The rafts were isolated after deposition and were kept under almost continuous observation. In many cases the act of hatching, which rarely lasted more than three minutes for the whole raft, was observed from start to finish. The timing of the incubation period began when the first eggs were seen to issue from the gravid female and this is the starting-point for the "maximum" figure in Table 2. Since oviposition was timed to completion, a second value, the "minimum", also appears in this table. Occasionally eggs hatched during brief absences of the observers, which increased the gap between minimum and maximum times. With one or two exceptions the difference between these two readings was 0.25 hour (15 minutes), which is approximately the time required for the female to oviposit a full raft.

In the absence of any temperature-control apparatus we had to be content with the normal insectary climate, which remained fairly constant at 28.1°C± 0.7°C and a relative humidity of over 90%.

The day after the larvae had hatched, the unembryonated eggs and unhatched dead embryos were counted.

Results

In all, 48 rafts were kept under sufficiently precise observation to merit reporting and discussion. The results are shown in Table 2. From these we concluded that the *incubation period* of the egg at 28.1°C± 0.7°C lies between 26.3 and 27.8 hours. By averaging the means of the minimum and maximum times and adjusting the relevant standard deviations according to the formula

$$(\{(SD_{min})^2 + (SD_{max})^2\}/2)^{1/2}$$

TABLE 2

INCUBATION PERIOD OF EGGS OF *C. P. FATIGANS* IN LABORATORY AT 28.1°C±0.7°C

Raft no.	Incubation period (hours)	
	Maximum	Minimum
1	27.33	27.08
2	27.25	27.00
3	26.75	26.00
4	26.75	26.00
5	26.67	26.42
6	26.67	26.42
7	26.75	26.00
8	26.67	26.42
9	27.25	27.00
10	26.67	26.00
11	26.67	26.00
12	27.25	27.00
13	26.83	26.58
14	26.83	26.58
15	26.83	26.58
16	27.01	26.58
17	26.67	26.42
18	26.83	26.58
19	26.75	26.50
20	26.70	26.45
21	26.78	26.53
22	26.75	26.50
23	26.58	26.33
24	27.00	26.75
25	27.33	27.08
26	27.25	27.00
27	27.33	27.08
28	26.95	26.70
29	26.82	26.62
30	27.03	26.78
31	26.92	26.67
32	27.92	27.67
33	28.17	27.92
34	27.83	27.58
35	28.17	27.92
36	28.00	27.75
37	28.17	27.92
38	27.92	27.67
39	27.75	27.50
40	27.75	27.50
41	28.17	27.92
42	27.75	27.50
43	27.75	27.50
44	27.90	27.65
45	27.82	27.57
46	27.87	27.62
47	27.63	27.38
48	27.85	27.60
Mean	27.26	26.95
Standard deviation	0.53	0.60

TABLE 3
NUMBER OF HATCHED AND UNHATCHED (DEAD EMBRYO OR NON-EMBRYONATED) EGGS FROM RAFTS OF *C. P. FATIGANS* OVA

Raft no.	Total no. of eggs	No. hatched	No. of dead embryos	No. non-embryo-nated
13	182	180	0	2
14	223	202	4	17
15	185	185	0	0
16	180	10	1	169
17	245	245	0	0
18	172	164	1	7
19	151	151	0	0
20	125	125	0	0
21	282	263	0	19
22	128	128	0	0
23	280	279	0	1
24	223	220	1	2
25	180	179	1	0
26	167	166	0	1
27	226	172	45	9
28	114	114	0	0
29	117	117	0	0
30	167	167	0	0
31	227	218	1	8
Total	3 574	3 285	54	235
Mean	188	173		
Percentage of total		92	1.5	6.5

we arrived at a single figure for the incubation period — namely, 27.11 $\pm$0.57 hours. This is lower than the value determined by Shriver & Bickley (1964), which was 28-32 hours at 85°F (29.4°C).

The over-all proportion of eggs that hatched was 92%, the unhatched eggs being made up of 1.5% dead embryos and 6.5% non-embryonated eggs (Table 3). The number of eggs that do not hatch varies in every collection of rafts deposited in the laboratory or collected in the field. We have shown elsewhere (de Meillon & Sebastian, unpublished) that the main cause of unembryonated eggs is lack of fertilization and have suggested that this fact may be used in assessing campaigns aimed at controlling the males. The cause of dead embryos is obscure and their number, as well as that of the unfertilized eggs in any given raft, is unpredictable. Shriver & Bickley (1964) apparently did not consider this aspect when they reported mortalities at different temperatures.

The *hatching period* was observed in several rafts from the start, when the first free-swimming larva appeared, to the end, when the vast majority had hatched. The start of the process was usually heralded by a sudden movement in the formerly motionless raft; close examination revealed one or two free larvae. Within seconds the raft began to break up and finally collapsed, with the appearance of a cloud of larvae, which for a moment or two remained motionless and appeared as a white nebulosity. The whole hatching process rarely exceeded three minutes.

PUPATION AND EMERGENCE OF *C. P. FATIGANS* AND THE DURATION OF LARVAL AND PUPAL LIFE

These subjects were investigated by Qutubuddin (1953), in a long series of experiments extending over a year. He found that there was " no appreciable variance in the pupal life of males and females ", that males emerge before females, that the sexes emerge in a 1 : 1 ratio, and that food and temperature do not affect this ratio. Unfortunately, he did not specify how often in 24 hours readings were made, but it seems that they were made daily or perhaps twice in 24 hours. Some of his findings do not agree with ours.

Haddow, Gillett & Corbett (1959) made a critical examination of the problem in *Aedes aegypti* and their results agree very well with our own reported here; they made hourly readings.

Material and methods

Our experimental material was derived from egg rafts deposited by *C. p. fatigans* either in the laboratory by day or in the field at night, in either case under conditions that permitted the oviposition time to be accurately determined.

The rafts were allowed to hatch in water containing our standard finely ground Spratts' Bonio dog biscuit. This was done because we had discovered in previous work that the withholding of larval food from first-stage larvae for some hours markedly prolongs subsequent larval life and hence the time from hatching to pupation.

Larvae were reared under standard conditions in

white enamel trays, and from just before the time when the first pupa appeared they were kept under continuous observation. As pupae appeared they were transferred singly to tubes and these were then also kept under continuous observation. By allowing the larvae to pupate in the trays before transferring them to tubes it was possible to see at a glance when pupae emerged. We found this method to be less laborious than isolating fourth-stage larvae and then having to examine all the tubes every time to see if any larvae had pupated (the method adopted by Haddow, Gillett & Corbett (1959)). The numbered tubes containing the pupae were placed on a slanted rack with a background of white paper. Since the pupae were isolated as they appeared, hatching occurred, with minor exceptions, in the same order, so it was sufficient to keep about a dozen tubes under constant observation at a time in order to determine the moment of emergence.

The mean temperature during the experiments was 28.6°C±0.8°C.

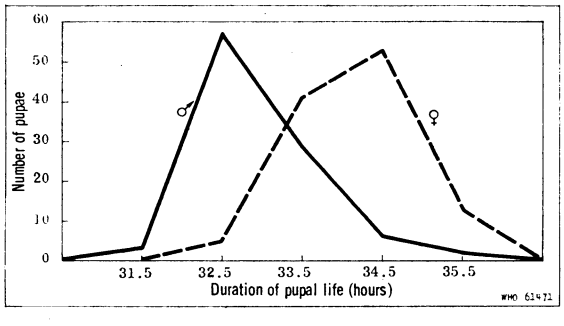
Results

Duration of the pupal stage. Fig. 1 summarizes the results obtained on the duration of pupal life, for males and females separately. The mean duration is 32.95±0.75 hours for males and 34.16±0.74 hours for females. The *F*-test indicates that the variances between the two sexes do not differ statistically ($P<0.05$). However, the difference between the means was found, by the *t*-test, to be significant.

It is therefore clear that females spend a significantly longer time in the pupal stage than males. This is in accordance with the results of Buxton & Hopkins (1927) and Haddow, Gillett & Corbett (1959) working with *Aedes variegatus* and *A. aegypti*, respectively, but contrary to Qutubuddin's (1953) findings with *C. p. fatigans* in Karachi.

FIG. 1

MEAN DURATION OF PUPAL LIFE OF *C. P. FATIGANS*



The pupating and emerging cycles. In this investigation we used two lots of larvae — namely, (*a*) those from ova deposited during the daytime and (*b*) those from ova deposited at night. Results obtained with larvae from eggs deposited during the daytime are shown in Fig. 2.[1] The rafts were deposited on 1 July 1965 between 10.45 and 11.00 hours. Several of the rafts hatched at 14.36-14.37 hours on 2 July and these were used in the experiment illustrated in Fig. 2.

There is no sign of a 24-hour rhythm. The rate of pupation and emergence under the present conditions simply increases to a peak in both males and females and then declines.

Correlation between duration of larval life and duration of pupal life. Since the males and females hatch at the same time from the raft and the former pupate first, the larval life of the male is clearly shorter than that of the female. It also appears that the durations of larval and pupal stages are directly related in both sexes; in other words, larvae that take a long time to pupate also take a long time to hatch. This phenomenon was also reported by Haddow, Gillett & Corbett (1959) to occur in *Aedes aegypti*. In view of this our data were analysed and it was confirmed that such a correlation does exist.

In Table 4 the hourly means of the duration of the larval and pupal stages are given. A scatter diagram in which duration of larval life is plotted against duration of pupal life shows a clear correlation between the two. Statistical analysis of the duration of the life of each individual larva and pupa revealed a highly significant positive correlation of $r=0.68$.

Duration of male and female larval life. The hourly means of the length of male and female larval life are given in Fig. 3. Analysis of the data gives the mean larval life of the male as 118.4±2.4 hours and that of the female as 135.3±4.4 hours. A *t*-test shows the difference to be highly significant.

Summary of duration of various stages of C. p. fatigans. In Fig. 4 we present a composite picture which displays graphically the time *C. p. fatigans* spends in each of the stages up to the emergence of the last female.

It must be emphasized that this figure summarizes the events that take place under certain conditions of temperature (28.6°C±0.8°C) and feeding. The durations

[1] The results obtained from ova deposited at night, although not entirely satisfactory, showed no marked differences from those obtained from eggs deposited during the daytime.

FIG. 2
PUPATION AND EMERGENCE IN *C. P. FATIGANS* OVA DEPOSITED AT 10.45-11.00 HOURS ON 1 JULY 1965

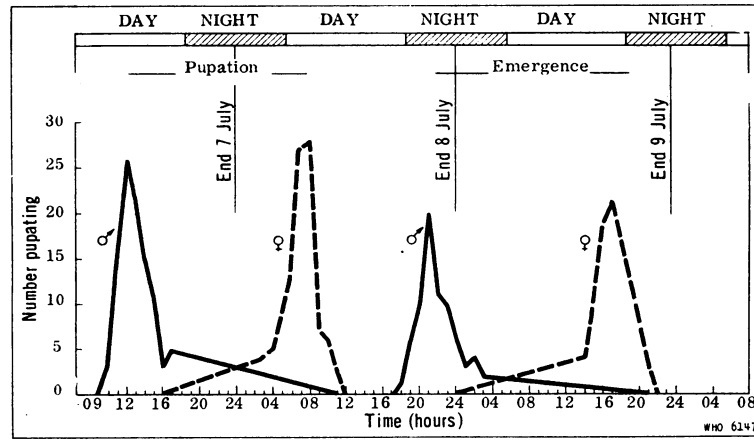


FIG. 3
MEAN DURATION OF LARVAL LIFE OF *C. P. FATIGANS*

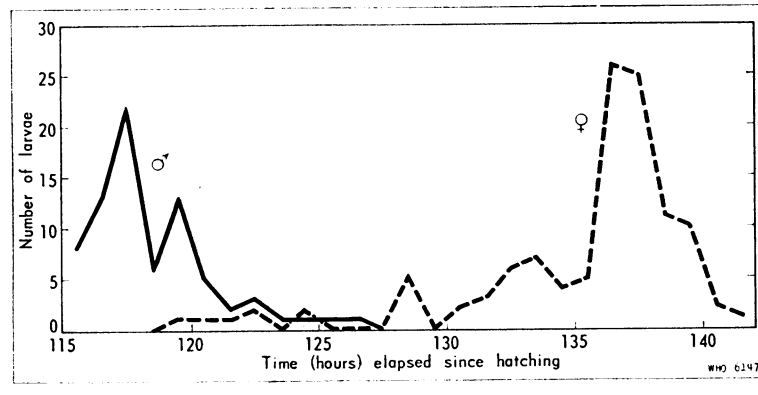


FIG. 4
DURATION OF VARIOUS STAGES OF *C. P. FATIGANS* UP TO EMERGENCE OF LAST FEMALE

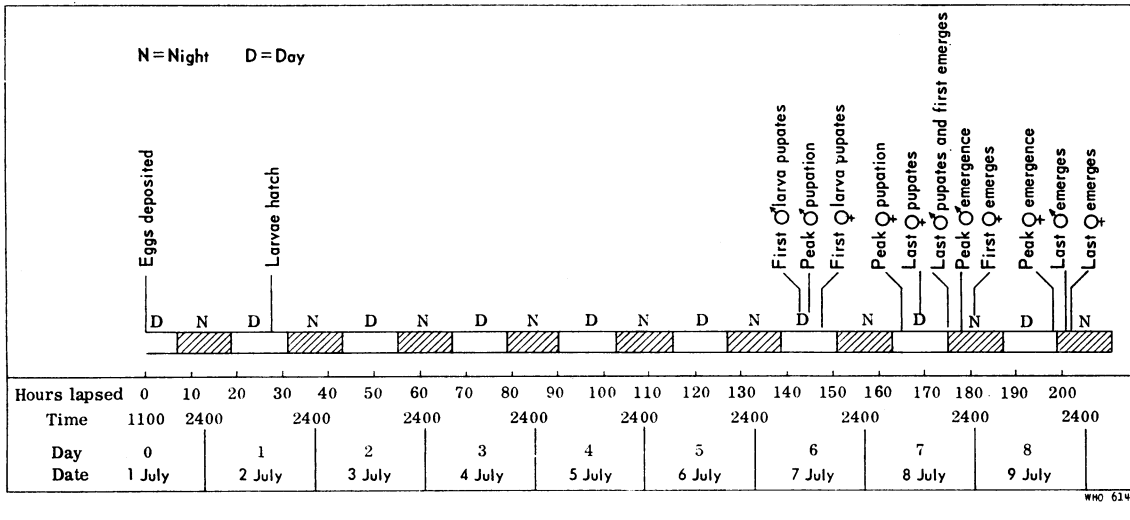


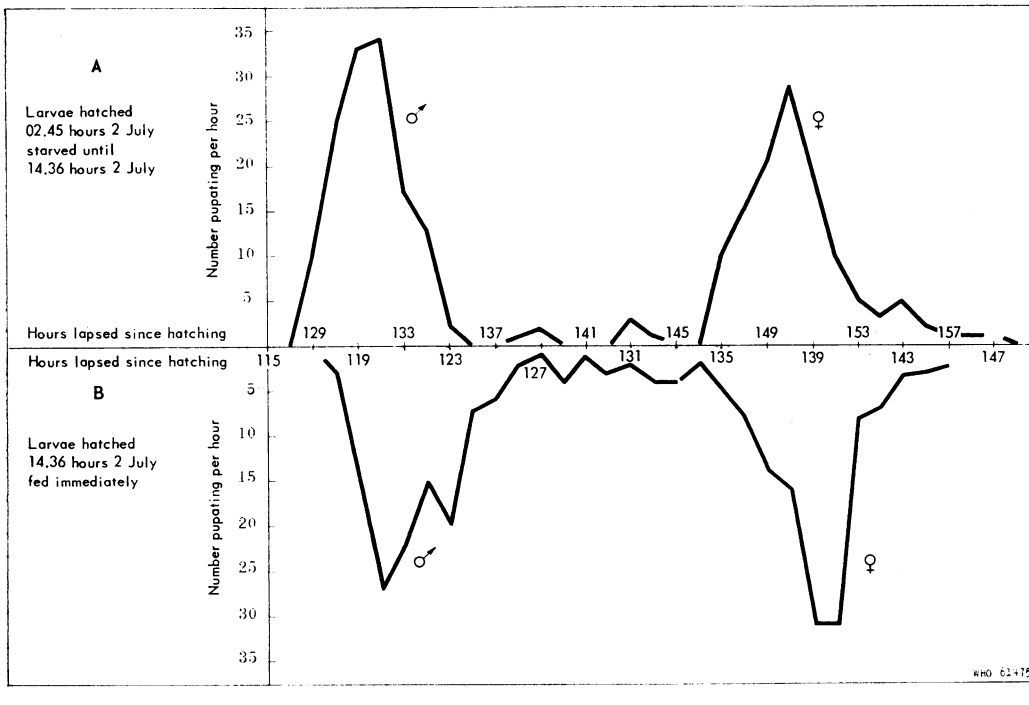
TABLE 4

HOURLY MEANS OF DURATION OF LARVAL AND PUPAL LIFE OF *C. P. FATIGANS*

No. of observations	Duration (hours) of		No. of observations	Duration (hours) of	
	Larval life	Pupal life		Larval life	Pupal life
	Males			Females	
8	115.5	32.6	5	128.6	33.9
13	116.3	32.7	2	130.2	34.3
22	117.4	32.8	3	131.7	33.6
6	118.3	32.4	6	132.6	34.7
13	119.5	32.8	7	133.7	33.9
5	120.4	32.9	4	134.3	34.3
2	121.5	33.1	5	135.8	34.0
3	122.1	33.1	26	136.8	34.2
1	123.0	33.4	25	137.8	34.2
1	124.8	34.2	11	138.2	34.8
1	125.3	33.8	9	139.5	34.4
4	126.5	33.5			

FIG. 5

EFFECT OF STARVATION OF FIRST-STAGE LARVAE OF *C. P. FATIGANS* ON DURATION OF LARVAL LIFE



of the egg and pupal stages are dependent on temperature only, as far as we know, but the duration of the larval stage is also greatly affected by the feeding conditions, as will be shown in the next section.

Effect of withholding food on length of larval life. The effect of withholding food on the length of larval life is a well-known phenomenon that can be demonstrated in a variety of ways. However, we were surprised to observe that the withholding of food from first-stage larvae during the first few hours of their life can have such a dramatic effect. One would be inclined to think that starvation in this early stage would be compensated for completely during the rest of larval life, but this does not appear to be so, as the following evidence shows.

During preliminary work on the pupating cycle, eggs were obtained from *C. p. fatigans* during daylight and at night. The interval between these ovipositions was 12 hours and the time of hatching was accurately determined. Larvae that hatched at night were left in ordinary tap-water until the next morning (12 hours later), when they were placed in a prepared breeding-tray. The larvae that hatched in the daytime were immediately put into a prepared breeding-tray. The times taken to pupate and emerge were duly recorded. Here we shall deal only with the duration of larval life, since our figures show that the duration of pupal life is not dependent on larval nutrition.

The results are presented graphically in Fig. 5, from which it can be plainly seen that larvae A, in spite of being about 12 hours older than larvae B, pupated at about the same time. In other words, the 12 hours of starvation after hatching were not compensated for during growth to the pupal stage, and larvae A behaved as if they had hatched at the same time as B, i.e., 12 hours later.

To obtain additional information we took some rafts all laid within an hour of one another at night and placed each in a tube by itself to hatch. Half the rafts, lot I, were allowed to hatch in tap-water only and the other, lot II, in tap-water containing dog biscuit. Twelve hours later, larvae from lots I and II were each put into a prepared breeding-pan and the time taken to pupate was noted. By the end of the sixth day after hatching, 195 of the 301 larvae from lot II (64%) had pupated. In lot I pupation began six hours later and by the end of the sixth day after hatching only 33 of 414 larvae (8%) had pupated.

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RÉSUMÉ

Les auteurs présentent les résultats d'une étude de laboratoire menée à Rangoon, Birmanie, sur la durée des stades préimaginaux de *Culex pipiens fatigans*. Cette durée est importante à connaître pour fixer l'intervalle entre des applications successives de larvicides. Etant donné qu'à Rangoon la température moyenne change peu au cours de l'année, il est possible de négliger l'influence éventuelle de ce facteur.

A la température de 28,1°C±0,7°C, la période moyenne d'incubation est de 27,11±0,57 heures. L'éclosion des larves mâles et femelles a lieu simultanément, mais la nymphose se produit plus précocement chez les premières et la durée de vie larvaire des mâles (en moyenne 118,4± 2,4 heures) est nettement inférieure à celle des femelles (en moyenne 135,3±4,4 heures). Le stade nymphal dure plus longtemps chez les femelles (34,16±0,74 heures) que chez les mâles (32,95±0,75 heures), à 28,6°C±0,8°C. Les phénomènes de nymphose et d'éclosion imaginale ne suivent aucun rythme de 24 heures. Dans les deux sexes, on note une corrélation entre la durée respective des stades larvaire et

nymphal: chez les larves qui mettent longtemps à se transformer en nymphes, l'éclosion imaginale est également retardée. Si l'on supprime la nourriture pendant quelques heures à des larves du premier stade, la durée de la vie larvaire est augmentée mais celle de la vie nymphale reste inchangée.

Ces observations sur la différence de durée des stades larvaire et nymphal suivant le sexe concordent avec celles qui ont été faites sur *Aedes variegatus* et *A. aegypti*, mais leurs résultats ne corroborent pas ceux d'études similaires effectuées sur *C. p. fatigans* à Karachi.

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