STUDIES ON THE BIONOMICS OF *CULEX SALINARIUS* COQUILLETT I. OBSERVATIONS ON THE CREPUSCULAR AND NOCTURNAL ACTIVITIES OF ADULT FEMALES [1]

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The mosquito, *Culex salinarius* Coquillett, occurs throughout most of the United States, but it reaches its greatest abundance in the Atlantic and Gulf Coastal regions, where it abounds in fresh, polluted and saline waters (Carpenter and LaCasse, 1955). Here it is reported to be the third most important pest species, according to Griffitts (1928). It is found as far west as Utah (Nielson and Rees, 1959), as far north as Nova Scotia (Twinn, 1945), as far south as Mexico (Vargas, 1956), and as far east as the Bermuda Islands (Williams, 1956).

In Delaware, as in other coastal states, the practice of impounding water on salt marshes to eliminate breeding sites of salt-marsh *Aedes* and to produce areas suitable for wildlife conservation has provided highly favorable conditions for this species; and isolations of eastern encephalitis virus from this species in the nearby state of New Jersey, as reported by Burbutis and Jobbins (1957), emphasize the need for more detailed information on its bionomics.

The object of the present study is t define more exactly the characteristics o its activities during the twilight and dar periods which were previously unknow It has been reported that females of C *salinarius* are not often diurnal bite (Snow, 1955, and Breeland *et al.,* 1961 Indeed, in all of our work among larg populations we were seldom attacked du ing the daylight hours.

Adults of this species have been capture in relatively large numbers by standa New Jersey light traps in the Atlantic an Gulf Coast States (Carpenter and LaCass *loc. cit.*). In Delaware, collections of high as 1114 females per night were r corded from light traps at Delaware City 1954. Headlee (1931, 1945) reported th *C. salinarius* was a night flier and that flies readily in the early evening. Math son (1944) stated that it bites huma readily and is active during the evenin This information suggested that bo light-trapping and human-biting rat could serve as criteria for the evaluation adult female activity.

Materials and Methods. A series of light trap studies were conducted on di ferent nights. Four were completed August, 1958 and the remainder in A gust, 1959. This month was selected b cause it is then that adult populatio commonly reach a high density in Del ware (Darsie *et al.,* 1953). In the latt year, they were supplemented on the sa nights by human-biting counts in order evaluate the two collecting methods.

The principal field study site was l cated about 2½ miles east of Delawa Bay on the Bombay Hook National Wil life Refuge near Smyrna, Delawa

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ıth the light-trap and human-biting data ·re collected on a dike separating two sh-water impoundments from each ıer. One, Raymond Pond, consisting of proximately 100 acres and the other, earness Pond, with nearly 560 acres, ›vided excellent breeding sites for *C. inarius*. The dominant plant species ›wing on the periphery of these ponds ·re *Typha* spp., *Phragmites communis* in., *Panicum virgatum* L., *Hibiscus* ›*scheutos* L., and *Echinochloa walteri* ursh) Nash. These plants furnished :ellent shelter for adults. Both ponds utted the tidal marsh, which was located ›ut 100 yards from the study sites. asses on the dike had been kept mowed :h summer so that there was no tall ;etation for at least 50 feet around each the collecting stations. The two sites ·re approximately 450 feet apart.

Adult trapping collections were begun minutes before and continued for three urs after sunset; during the latter period consecutive 15-minute collections were ıde. On two nights, light trapping and ing counts continued throughout the ;ht and ended during morning twilight. standard New Jersey mosquito light p, equipped with a 25-watt bulb and wered by a 3000-watt portable genera- , was utilized. A pint jar with sodium ınide as the killing agent was attached the bottom of the trap from which mos- itoes were transferred to pill boxes at end of each 15-minute period. Air ıperature, relative humidity, and light ensity were recorded at the midpoint of h 15-minute period. Temperature was ermined with a Taylor maximum and nimum thermometer and relative hu- dity by a Humidiguide hygrometer. th instruments were mounted on a post eet above the ground. A Weston pho- neter (model 650), with a sensitivity of foot-candle was employed for the meas- ment of light intensity changes. Using technique of Dyson-Hudson (1956), ht reflected from a one-foot square white d placed horizontally on the ground s measured to determine the intensity. sts were made only when the wind was not more than an estimated 5 miles per hour, and only in clear weather.

The biting collection method employed was modified from that of Beadle (1959). Whereas light-trapping continued throughout the whole 15-minute period, human-biting counts were taken only during the first five minutes of each 15-minute period. It did not seem practicable to make accurate collections for a longer continuous period with such high numbers of mosquitoes. Systematic human-biting collections were accomplished while the investigator was seated upon a stool. They were made with a mechanical aspirator described by Murphey (1962), from one leg leg exposed to the knee. The aspirator was fashioned from a two-celled, battery operated, flashlight-type vacuum cleaner. Sufficient illumination for after-dark collections without disturbing the search of the female mosquitoes for blood, was provided by a flashlight equipped with a red lens (Corning Glass Filter, No. 243–978), which transmitted light of wave lengths between 6720 and 6869 Angstroms. Available information on color perception in insects suggested that this would not disturb female mosquitoes in their search for blood. Wigglesworth (1947), indicated that insects, in general, are insensitive to red light, and Roeder (1953) stated that at the long end of the spectrum the maximum effective wave length for most insects is about 6500 Angstroms.

For the purpose of these experiments, the astronomical summer light conditions, such as the time of sunset and duration of twilight, were derived from the tables of Eckert and Clemence (1946). There is some confusion about the term "darkness" as used by Beadle (1959) and Breeland *et al.* (1961). The time at which darkness begins and ends, as used here, is taken from Eckert and Clemence and is defined as the time when the center of the sun's disk is 18° below the horizon. In the analysis of the tests, the collection figures were reduced to a least common denominator of adults captured per minute.

Results. The data summarizing the results of the 12 light-trap experiments are

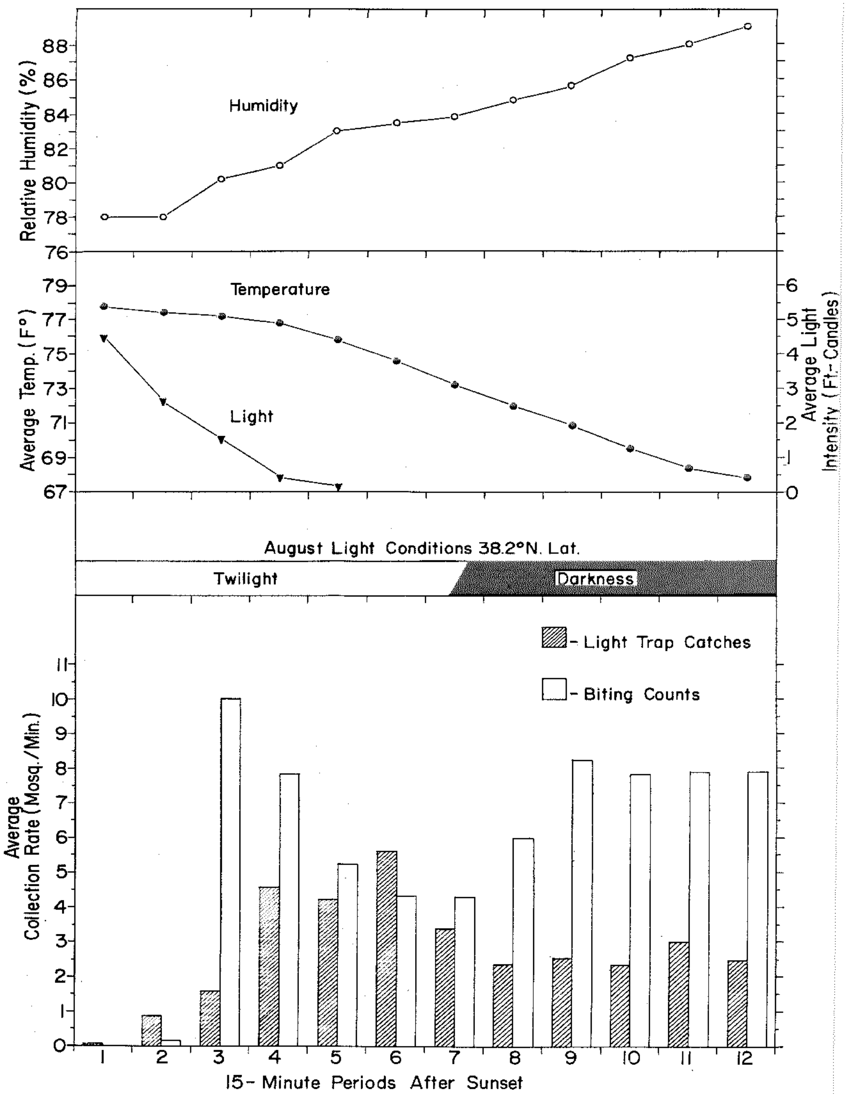
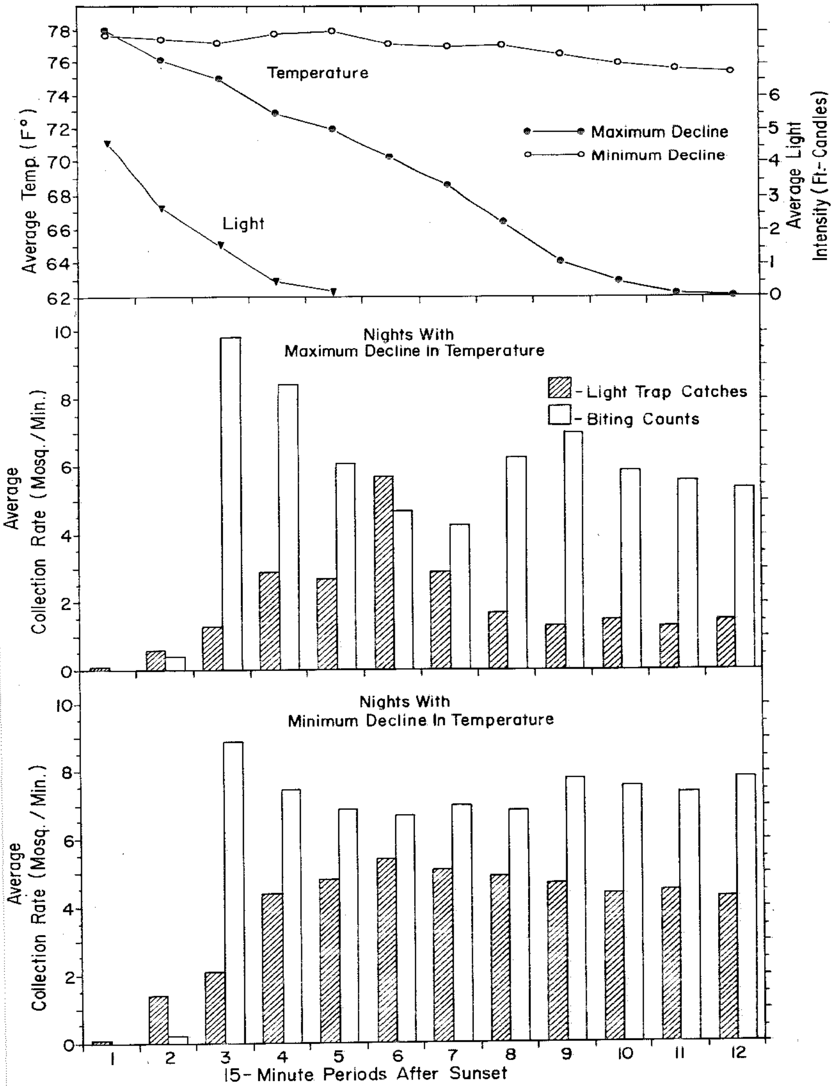


FIG. 1.—Light-trap and human-biting collections of *Culex salinarius* during successive 15-mi periods after sunset, Bombay Hook, Delaware, August, 1958 and 1959, showing average number females captured per minute, light intensity, temperature and relative humidity.



IG. 2.—Light-trap and human-biting collections of *Culex salinarius* during successive 15-minute iods after sunset on two nights with minimum decline and two nights with maximum decline in perature, Bombay Hook, Delaware, August, 1959, showing average number of females per minute. t intensity and temperature.

presented in Figure 1. The information was derived from a total of 5946 captured females. Light-trap activity began during the first 15-minute period following sunset with a relatively small collection rate of 0.05 female per minute. The first substantial increase occurred during the fourth 15-minute period when the midpoint light intensity averaged 0.4 foot-candle, temperature averaged 76.8° F. and the relative humidity, 78 percent. Figure 1 shows that the highest numbers were trapped during the fourth, fifth and sixth 15-minute periods.

During the latter period a peak collection of 5.6 adults per minute was taken. Light intensity during the 15-minute period averaged less than 0.1 foot-candle, while the temperature had declined to 74.6° F. and the relative humidity had risen to 83.5 percent. Following this peak and proceeding into darkness a sharp decline in females trapped occurred, reaching an average of 2.3 adults per minute in the eighth period. Activity continued at approximately this rate during the remainder of the three hours.

The results of the eight human-biting trials, in which a total of 2794 females were collected, are also included in Figure 1. In contrast to the light-trap activity, the biting collections rose more sharply to an early peak of 10 females per minute at the third 15-minute period after sunset. At the same time, the temperature declined less than one degree to 77.2° F., and the relative humidity increased by 2 percent to 80.2 percent.

Following the peak period there was a gradual decline in biting activity to a low of 4.3 females per minute during the seventh period. At that time light intensity had declined to an average value of less than 0.1 foot-candle. Following the onset of complete darkness, activity rate ascended again and was sustained at a rate of 6.0–8.2 adults per minute through the remaining test intervals.

The data from the light-trap collections and biting rates for the two nights when temperatures remained highest, and two nights when they were lowest are p sented in Figure 2. This comparis shows that, after natural light has ceas to be an influence, activity, as measured either trapping or biting, was direc related to temperature.

Average data for the two all-nig light-trap and human-biting collections presented in Table 1. The trap catc decreased from the peak in the sixth minute period to a plateau of 6 to females per 15 minutes, with minor fluc ations, which lasted until one hour bef morning twilight, at which time mo ment into the trap ceased.

In the human-biting there were t activity peaks, an initial one at the th 15-minute period and the other extend from the ninth to the twelfth period. S sequently the activity followed essenti the same pattern as the light trap exc that the decline throughout the night v more gradual.

During the operation at both collect sites these other species of mosquitoes w collected: *Aedes cantator* (Coq.), *A. licitans* (Walk.), *A. vexans* (Mei *Anopheles crucians* Wied., *An. qu rimaculatus* Say, *Culex pipiens* L., *restuans* Theo., *C. territans* Walk., *M sonia perturbans* (Walk.) and *Psoroph confinnis* (L.-A.).

The combined population attacking human bait was at times extremely hea The highest single collection occurred tween 2030 and 2035 on August 6, 19 when 160 females were captured. It cluded 3 species of *Aedes,* 2 of *Anoph* and 2 of *Culex.* After *C. salinarius* next most abundant species was *A. licitans.*

Discussion. The flight periods of m crepuscular and nocturnal insects closely related to a critical light inten (Barker, 1961). The most signific studies leading to the current knowle concerning the response of such insect changes in light intensity were condu with the codling moth, *Carpocapsa p onella* (L.), by Collins (1934) and Col and Machado (1935, 1943). Tasl

BLE 1.—Light-trap and human-biting collections of *Culex salinarius* during successive 15-minute periods following sunset for two all-night tests, Bombay Hook, Delaware, August, 1959

riod fter nset	Aver. No. per night		Mean temp. °F.	Mean percent R. H.	Period after sunset	Aver. No. per night		Mean temp. °F.	Mean percent R. H.
	Light trap	Human bait				Light trap	Human bait		
1	0	0	77.9	78.1	20	9.5	14.5	66.6	91.6
2	11.5	0	77.3	78.0	21	8.0	15.5	66.0	92.0
3	28.0	53.5	77.2	80.3	22	11.0	13.5	65.5	92.0
4	42.5	38.5	76.7	81.0	23	7.5	14.0	65.0	92.2
5	43.5	20.0	75.7	83.0	24	8.5	12.0	64.7	92.4
6	68.0	15.0	75.2	83.6	25	9.5	13.0	64.4	92.5
7 *	23.5	15.0	74.7	83.8	26	9.0	11.0	64.2	93.0
8	17.0	29.5	74.2	85.0	27	10.5	10.5	64.1	92.0
9	18.0	45.5	73.5	85.7	28	8.5	13.0	63.9	93.4
10	18.5	45.0	72.8	87.0	29	7.5	9.0	63.8	92.0
11	28.5	44.5	72.4	88.0	30	6.0	8.5	63.7	93.0
12	21.5	45.5	71.8	88.9	31	7.0	8.0	63.6	94.2
13	20.5	31.0	71.2	89.0	32	5.0	4.5	63.5	93.0
14	19.5	28.5	70.4	89.4	33	3.0	1.0	63.5	94.1
15	13.5	23.5	69.7	89.7	34	2.0	0.4	63.4	93.9
16	14.0	20.0	69.2	90.0	35	1.0	1.0	63.5	92.0
17	13.0	18.0	68.6	91.0	36 **	0	0	63.4	89.0
18	14.0	16.0	67.7	91.3	37	0	0	63.6	87.0
19	9.0	17.5	67.2	91.4	38	0	0	63.7	83.0

* Complete darkness occurred mid-way in this period.
** Morning twilight began in this period.

961) has summarized the results of these udies. Normally, the moths are at rest ıring the day. As evening approaches, ey initiate activity at a light intensity of ›out 30 foot-candles. This period of ovement was found to be associated ith the adaptation of the compound e to light magnitude changes, which as studied in detail by Collins and achado (1935). They found that the igration of iris pigment, which is as- ciated with a critical light intensity 30 ft.-candles), functions to regulate the nount of light impinging on the retina. his process conditions the response of ie moth to light.

It is possible that a similar process takes ace in crepuscularly and nocturnally tive Culicidae. There is evidence from ır tests that the light intensity condi- oning evening flight in *C. salinarius* was the range of 5.5 to 3.8 foot-candles verage 4.5 ft.-candles), just as 30 foot ındles activated the codling moth. Sup- ›rt of this view lies in the fact that no *C. salinarius* were captured either in the light trap or on human bait in natural light of greater intensity.

The two collecting methods indicated two different types of mosquito response, a fact recognized by Snow and Picard (1954) and illustrated in Figure 1, in which the peak of light-trap entrance occurred later than that of human biting. Therefore, results of the two methods will be considered separately.

Light Traps. It is believed that the data reported herein have established a time schedule for the light-trap response of *C. salinarius* following sunset. As the intensity of natural light declined, the light produced by the 25-watt frosted bulb in the light trap was progressively less masked and gradually became effective as an attractant. Natural light ceased to be a factor during the seventh 15-minute period following sunset with the onset of complete darkness, but the precise point at which it no longer was influencing *C. salinarius* activity is not known. In lepidopterous insects, *Diataraxia* and *Acronycta*, the threshold is reported to be below 0.5 foot-candle (Way and Hopkins, 1950). From our data it appears that it might be in the same range for this mosquito species, see Figure 1.

The interpretation of results on the pattern exhibited by *C. salinarius* is necessarily restricted to the salt-marsh habitat previously described, and the response may be different in others. However, Huffaker and Back (1943) recorded a similar pattern in fresh-water swampland in Northern Delaware, which was not near any typical salt-marsh breeding areas.

Figure 2 contains the results of the analysis of temperature as a factor influencing the movement of *C. salinarius* females. During the first six periods there appears to be little effect exerted by temperature. The peaks on both the nights with minimum and maximum declines occurred in the sixth period and were approximately of the same magnitude, despite the fact that on the nights of lower temperatures there was a drop of 7.7° F. up to that time, as compared with only 0.6° F. depression on the warmer nights. Beyond the sixth period the reduction in mosquitoes trapped was directly proportional to the decline in temperature. On nights of maximum decline the temperature difference from the sixth to the twelfth period was 8.3° F., while during minimum decline it amounted to only 1.8° F. Considering the sixth period catches as 100 percent, there was a 74 percent decrease in mosquitoes trapped in the former case, whereas only a 20 percent drop in the latter instance. The data also indicate that there is a critical temperature below which activity is markedly lessened, since according to Figure 2, the number trapped fell below an average of two per minute when temperatures decreased to 66.4° F. and lower. Breeland *et al.* (*loc. cit.*) state that attempts of this species to feed on blood are inhibited when the "low 50's" (50° F.) are reached; however, Headlee (1932) noted that mosquito flight was suppressed by temperatures below 60° F.
Moreover, it has been pointed out Bradley and McNeel (1935) that in Flori temperatures below 70° F. greatly reduc mosquito activity. So this factor is a fu tion of meteorological conditions as th vary with latitude changes.

Human-biting Counts. As illustrated Figure 1, the biting activity was initiat during the first 15-minute period when t light intensity measured 4.5 foot-candl The rate rose sharply to a peak of adults per minute in the third period, which the average light intensity measur 1.5 foot candles. Temperature a humidity changes, which decreased or 0.7° F. and increased only 2 perce respectively, appear to be too insignifica to have exerted any influence.

Snow (1955) reported that in Tenness female *C. salinarius* characteristica migrated at dusk from diurnal resti places. He concluded that light was t activating and deactivating influence f diurnal and nocturnal populations of b ing Diptera (including *C. salinariu* Also, Snow *et al.* (1958), in South C olina, observed a striking increase in t biting rate of *C. salinarius* during the thi 15-minute period after sunset, which agre with the Delaware data.

According to Beadle (*loc. cit.*) the pe biting activity of *Culex tarsalis* Coq. beg 30 minutes after sunset and continued f one hour with the largest catches comi in the fourth 15-minute period after su set. While the peak biting activity beg at the same time in these tests with *salinarius*, it was not sustained but dropp to a low during the seventh period a rose to a secondary peak in the nin period, 120 minutes after sunset. This p tern exhibited by *C. salinarius* may correlated with the activity of their m commonly available hosts in nature; f example, the presence of diurnally acti birds in nearby roosts and rookeries or t initiation of movement by noctur animals. Also it is possible that the *salinarius* which had emerged from pup inhabiting the nearby fresh-water pon found the bait first and produced the fi ak, then the females from the more tant salt-marsh breeding areas arrived er to form the second peak.

The influence of temperature on the bit- rate was also studied (see Figure 2). cept for the initial peak, the biting ivity was depressed on the two nights h maximum decline, although the luction was not nearly so pronounced as the light-trap samples. Considering the ak third period as 100 percent, there s a 44.9 percent decrease in mosquitoes ring a 13° F. drop from the third to elfth periods. When temperature de- ne was minimal (1.9° F. in the same n) only an 11.2 percent drop was expe- nced. The secondary peak is much less ticeable on the warmer nights, due to tained activity. Beadle (*loc. cit.*) re- rted a similar prolongation of peak bit- activity in *C. tarsalis* on nights when nperatures remained relatively high.

The influence of temperature on human- ing rate was further evident as a result the two all-night samplings; see Table There was a gradual decline after the elfth period, although masked somewhat minor fluctuations. The downward nd can be seen better by grouping the iods from the 17th to the 28th into 30- nute units (thus, the 6 units averaged .0, 16.0, 14.5, 13.0, 12.0 and 11.7 nales). This is apparently related to the vering of the temperature and rise in ative humidity as the night progressed. ie light-trap catches do not exhibit nearly pronounced an effect. However, by both npling methods there was a cessation *C. salinarius* female activity at morning ilight.

The biting counts were continued only five-minutes during each 15-minute riod, but even so, on a per minute basis ore female *C. salinarius* were captured human bait than in the light-trap erated for the full 15 minutes, as shown Figure 1. The data indicate that the man was more attractive to this species an was the light in the trap.

In order to emphasize the probability of aks occurring in the periods indicated, it can be stated that in 10 of the 12 nights sampled, as presented in Table 1, the largest numbers were trapped during the sixth 15-minute period after sunset. Similarly, on all eight nights the biting count peaks occurred in the third period, as already mentioned.

In surveys of population size of a particular pest or vector species of mosquito, the magnitude of the catch as measured by a given sampling method is subject to the influence of meteorological factors, in addition to the actual density, as suggested by Morris (1960). This is particularly true when a sampling device, such as the New Jersey mosquito light trap, is operated for relatively long periods of time. In a period by period analysis of our data, peaks of *C. salinarius* occurred consistently in certain intervals, and it suggests the possibility of limiting the trapping (or human biting counts) to the peak periods after sunset indicated, in order to measure more accurately the relative population indices. It appears that within limits this would eliminate the effect of temperature on the size of the long interval catches. For example, on August 10, 1959, when temperature decline was minimal, the total number collected in the trap during the 12 periods was 757 female *C. salinarius*, and 88 were trapped in the peak sixth period (see Table 1), while on August 26, 1959, when temperature decline was maximal, the total amounted to only 402 females. In the sixth period 108 were collected, so that the trap collections for the sixth period alone, therefore, may be a better estimation of the population in the area than totals for the 12 periods, or even for all night.

CONCLUSIONS. 1. *Culex salinarius* females do not begin their daily flight activity until immediately after sunset, and it is terminated before sunrise.

2. Peak period of human biting occurs during the third 15-minute period following sunset, while the light-trap entrance reached a maximum later, during the sixth period. These observations apparently are correlated with natural light intensity.

3. The results indicate that the peak period of activity as measured by light-trap or human-biting collection was prolonged on nights when temperature decline was minimal.

4. Human bait is more attractive to *C. salinarius* females than the standard New Jersey light trap.

5. In order to determine more accurately population size, limiting the sampling to the peak activity periods is suggested.

6. It seems reasonable to assume that, in the control of adult *C. salinarius* with insecticides, applications should be made during the evening twilight period of the day, because this is the time of maximum flight activity.

7. Sustained human biting throughout the night implies a possible long daily interval when disease organisms could be transmitted by this species, and the need for continual protection during that period.

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A SIMPLE TECHNIQUE FOR OBTAINING STANDARD NUMBERS OF NEWLY HATCHED MOSQUITO LARVAE

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Introduction. The mosquito *Aedes aegypti* L. is reared in many laboratories for studies in various fields, because of the relative ease with which it can be reared, and the important advantage that its eggs can be stored for several months. The key to success in obtaining the maximal number of normal-sized adults from a given number of newly hatched larvae is a proper ratio between the number of larvae in a rearing container and the amount of food given to the larvae (Shannon & Putnam 1934, Bar-Zeev 1956). If, for a given number of newly hatched larvae, there is too much food, mortality of larvae and pupae will result, mostly due to scum formed on the water surface (Christophers 1960). If there is too little food, the time from first to final pupation will be prolonged; many larvae which have not yet pupated will be lost, unless they are separated (Bar-Zeev & Galun 1961), and placed in fresh water with food. Furthermore, the resulting adults will be small (Bar-Zeev 1956). If the number of larvae per breeding container can be kept constant, the optimal amount of food for this number of larvae can be determined.

In mosquito investigations, it is important to rear the larvae under identical conditions and to obtain adults of uniform size. It has been shown by Harrison (1952) that small house flies are more susceptible than large ones to DDT deposits. This may also be true of mosquitoes, not only in regard to susceptibility to insecticides but also to a number of other factors.

A survey of the literature shows that the number of larvae given per breeding container is either counted (Hartzell *et al.*, 1958) which is time consuming, or estimated by eye by various means (Trembley 1955, McKiel 1957, Christophers 1960 and Kirkwood 1961) which obviously yields somewhat unreliable results. In this study various techniques were tested in order to achieve a more satisfactory method. The following has proved to be the most suitable.

Materials and Methods. Eggs were submerged in clean water in a jar in the afternoon and left overnight (without adding food) to allow most larvae to hatch. The contents of the jar were then filtered through cloth held below a 40-mesh metal screen. The latter allowed the larvae to pass but retained most debris (egg shells, dead mosquitoes, etc.). The larvae were retained on the cloth and were then transferred to a beaker containing a little water, and filtered again through cloth placed in a 7 cm diameter funnel the stem of which had been cut off. Washing with a small quantity of water serves to concentrate the larvae at the bottom of the funnel, which is then placed on top of a beaker