earlei. Females emerge from the burrows in spring when the surface soil becomes warmer than the subsoil. Survival of mosquitoes in burrows depends greatly on the type of burrow, amount of snow cover, and exposure to winds during the winter.

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OVIPOSITION OF THE MOSQUITO *CULEX TARSALIS* IN RESPONSE TO LIGHT CUES[1]

DANIEL LOGEN[2] AND ROBERT F. HARWOOD
Department of Entomology, Washington State University, Pullman

The problem of cyclic oviposition in mosquitoes has been investigated before (*Aedes aegypti,* Haddow and Gillett, 1957; *Aedes africanus,* Gillett and Haddow, 1957; *Aedes apicoargenteus,* Haddow, Corbet and Gillett, 1960; and *Taeniorhynchus fuscopennatus,* Haddow and Gillett, 1958). In each case a clearly defined oviposition cycle was discovered; diurnal for the three species of *Aedes* and nocturnal for *Taeniorhynchus.* Furthermore, these rhythms are circadian, being maintained for some time under constant light or dark conditions.

The experiments described in this paper were conducted in an attempt to determine the nature of the relationship between photoperiod and oviposition in the mosquito *Culex tarsalis.* As soon as the presence of a daily rhythm under various light conditions was confirmed, particular emphasis was placed on the possibility of an internal rhythm governing oviposition.

MATERIALS AND METHODS. A strain of *Culex tarsalis* originating from Washington was used. For each experiment, egg rafts were taken from the stock culture and reared in incubators where temperature and light conditions were controlled. The pupae were transferred to another incubator which contained a screen cage and the egg collecting apparatus. This consisted of 13 small water-filled jars placed in a circle, above which rotated a disc with a single hole of the same diameter as a jar. Thus only one jar was uncovered at any one time. Rotation of the disc by a motor was controlled by an automatic timer. At each position a protuberance contacted a microswitch stopping advancement to the next jar until activation by the timer. After a period of as long as 2 days, (using 4-hour intervals) the jars were removed, the egg rafts counted and discarded, and the jars replaced.

Food supplied to the larvae was based on herring meal, (Harwood and Halfhill, 1964). Adults had a 10 percent sucrose solution available constantly, and for each

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oviposition experiment a chicken was made available for at least two overnight periods of 12 to 15 hours.

In order to save time, all experiments except one noted below were conducted at 30 C.; at this temperature the larval period was 6 to 8 days.

When necessary, observations in the dark were made using a red safety light; this seemed to have no effect on the results.

Results

Experiment 1. Oviposition in a 24-Hour Cycle of 16 Hours Light and Eight Hours Dark (16L:8D, Figure 1a). Observations were made for a period of 16 days, with the number of egg rafts recorded for each 4-hour interval. Of the 51 egg rafts recorded, 20 were deposited in the first 4-hour interval of light and 21 in the first 4-hour dark period. The resulting graph shows a well defined oviposition rhythm with two equal peaks occurring daily.

Experiment 2. (12L:12D, Figure 1b). Four-hour collections were made for 9 days during which 90 egg rafts were deposited. The results show two well defined peaks, but with the "morning" peak substantially higher than the "evening" peak.

Experiment 3. (8L:16D, Figure 1c). Observations were made for 9 days. The results were very similar to those in Experiment 2, except that the peak in the first light period was considerably higher than the peak in the first dark period.

Experiment 4. (24L:0D). When the larvae were reared in constant light and the oviposition test was also conducted in constant light, essentially no oviposition was observed. The little oviposition that did take place was randomly spaced throughout the 9-day observation period.

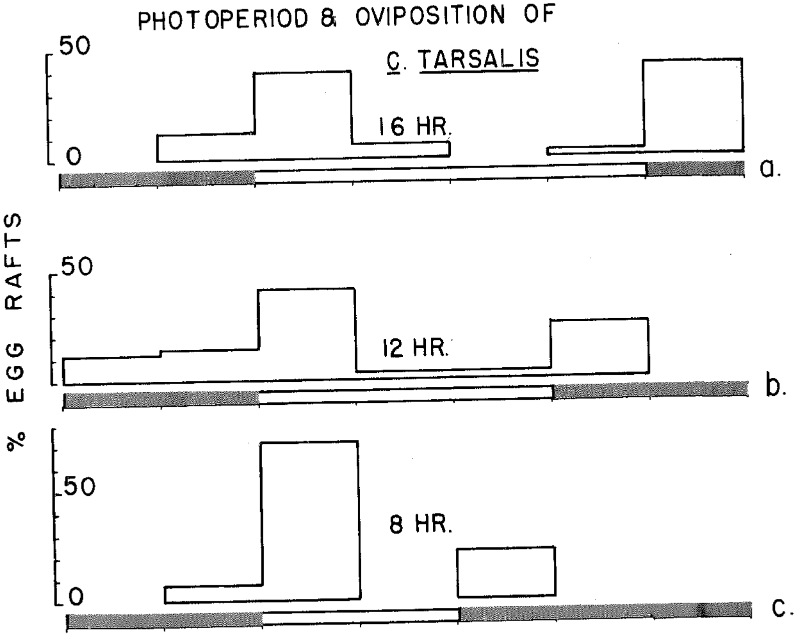

Fig. 1.—Oviposition of *Culex tarsalis* in response to light and dark cues. Each 24-hour period is subdivided into 4-hour units. The heavy black line at the base of each experiment indicates the scotophase, the open section the photophase.

Experiment 5. (0L:24D). As in Experiment 4, the amount of oviposition was very much below normal. In the 13 days of observation no rhythmical trend was observed in the limited oviposition.

In an attempt to determine whether the oviposition cycle is completely dependent on the light cues, or if an internal element is present, experiments were conducted in which all light cues were suspended after a definite rhythm was established. In these experiments, oviposition ceased immediately, regardless of whether the experiment was left in constant light or dark. If the light-dark cycle was resumed two or three days later, the oviposition cycle was also resumed, just as if nothing abnormal had happened. Furthermore, it made no difference if the cues were brought back out of phase. For instance, in one experiment an oviposition cycle was established using a 12-hour photoperiod with the photophase being from 08:00 to 20:00. The experiment was left in constant light for two and one half days, then a 12-hour photoperiod was resumed, but with light and dark exactly reversed with respect to previous training. An oviposition rhythm was immediately assumed, with the higher peak still occurring in the first light period. This is not too surprising, since there was an exact reversal of light and dark. But even when the cues were restored 8 hours out of phase, there was a resumption of the normal oviposition rhythm. This appears to be rather conclusive evidence against the existence of an internal rhythm controlling oviposition.

DISCUSSION. As noted for *Aedes aegypti* (Gillett, Haddow and Corbet, 1959), it is clear that alternating light and dark are necessary for an oviposition rhythm to occur in *Culex tarsalis.* Furthermore, in all experiments conducted, the change in light conditions seemed to trigger oviposition, with more egg rafts deposited just after the change from dark to light. In one experiment, the 4-hour periods in which the two peaks occurred were subdivided into units of one hour. In every case all oviposition was completed within the first hour. This suggests an immediate response to the change in light conditions and appears to coincide with general flight activity of both sexes observed under these conditions. Unlike *Aedes aegypti,* after training to a light-dark cycle the oviposition rhythm does not continue under constant light or dark conditions.

To determine whether or not temperature has an effect on the oviposition rhythm, an experiment was conducted in a normal 12-hour photoperiod at 22 C. instead of 30 C. No changes in the oviposition rhythm were detected. This temperature independence agrees with most observations on circadian rhythms (Pittendrigh, 1954; Bruce and Pittendrigh, 1957), though the rhythm observed here is not circadian.

Predominance of oviposition in the first peak when light comes on, under conditions of short photoperiod, may relate to expected temperature conditions in the natural environment. It seems reasonable that night temperatures would be too low for general activity at a time when day length is short, therefore oviposition predominates when light comes on because in nature this is the period when temperature would be sufficiently high for general flight activity and associated behavior such as oviposition.

SUMMARY. In a photoperiod of 8, 12, or 16 hours, the mosquito *Culex tarsalis* shows a regular, temperature-independent oviposition rhythm with peaks occurring in the first hour of light and the first hour of dark. When light and dark are changed, there is usually a corresponding change in the oviposition rhythm. No rhythmical behavior is present in constant light or constant dark and there is no evidence for an endogenous rhythm even after rhythmic response to light and dark cues. Predominance of oviposition in the first peak when light comes on, under conditions of short photoperiod, may relate to expected temperature conditions in the natural environment.

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THE EFFECT OF PHOSPHORUS^{32} ON THE FECUNDITY OF *AEDES AEGYPTI* (L.) AND ITS USE IN DETERMINING BLOOD MEAL VOLUMES

GORDON F. BENNETT[1]

INTRODUCTION. The measurement of quantity of blood ingested by blood-feeding insects has been the subject of study for some time, most of the studies employing a gravimetric technique. Christophers (1960), in his monograph on *Aedes aegypti,* has summarized many of the problems associated with such studies, concluding that fluid loss from the mosquito during and immediately following engorgement was a major source of error. Boorman (1960) used isotope-labelled blood to avoid some of the problems associated with gravimetric methods, particularly those concerned with the time at which mosquitoes were weighed following feeding.

The availability of the isotope phosphorus^{32} in this laboratory enabled us to apply Boorman's technique (with modifications) on simuliids (Bennett 1963) and to study *Aedes aegypti* in a similar manner. The work reported herein is a comparison of the results obtained by a gravimetric method and by use of isotope-labelled blood. In the course of these studies, casual observation suggested that mosquitoes fed on isotope-labelled blood laid fewer eggs than expected. This aspect was further studied and the results are reported herein.

MATERIALS AND METHODS. The mosquitoes used in this study were obtained from a colony of *Aedes aegypti* maintained in this laboratory. Mosquitoes used in any one experiment were taken from one rearing cage only, and were 5 to 6 days of age when used. Following feeding, engorged mosquitoes were maintained in small cages and provided with a sugar and water diet. The mosquitoes laid their eggs on pieces of wet filter paper placed in small crystallizing dishes. The host animals used throughout were domestic ducks and chicks obtained commercially.

Phosphorus^{32}, in the form of phosphoric acid ($H_3P^{32}O_4$), was obtained from the

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