# A CIRCADIAN PATTERN IN THE RESPIRATION OF LARVAE OF THE MOSQUITO *CULEX PIPIENS*[1]

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INTRODUCTION. Circadian rhythms have been demonstrated for such activities in the adult mosquito as flight (Jones *et al.*, 1966) and feeding (Haddow, 1964). Probably because of the difficulties in quantification, there has been little work done on daily rhythms of immature forms other than their effect on pupation (Nayar, 1967). Olifan (1947) suggested the possibility of diurnal metabolic rhythms but did not investigate it. This study of endogenous influences on larval respiration was initiated to determine optimal time for recording respiration as part of a more comprehensive metabolic study.

MATERIALS AND METHODS. This strain of *Culex pipiens* was obtained from the laboratory of Dr. James B. Kitzmiller, University of Illinois, who originally obtained it from Allerton Park near Monticello, Illinois in 1961. The colony was maintained under the described conditions for more than six months before the experiments were begun. The insectary was maintained at a relative humidity of $70 \pm 5$ percent and at a temperature of $27° \pm 1°$ C. The room was illuminated on a 15L9D photoperiod schedule, the first and last hour of which were subdued. These conditions simulate the midsummer environment of central Illinois. Larvae were reared in 22 cm x 32 cm enamel pans filled to a depth of 2 cm with dechlorinated water at room temperature. Because of evaporation, the actual rearing temperature was 25° C. Progeny of one egg raft were reared in each pan. They were fed daily a finely powdered mixture

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of one part brewer's yeast, two parts Kellogg's Concentrate, two parts Purina Lab Chow, and two parts wheat germ. Pupae were transferred daily into a bottle of water which was then placed in the colony cage. The adult breeding cage measured 0.4 $m^3$. Cotton soaked honey was always present as a source of carbohydrate. Bound pigeons were placed in the cage about every third night. A pan of water was kept in the cage for egg deposition. Eggs were removed daily.

A Gilson GR-14 differential respirometer (Gilson, 1963; Umbreit *et al.*, 1964) was used to determine respiration of the larvae. The water bath temperature was maintained at 25° ± 0.1° C. Filter paper containing 0.2 ml of 10 percent KOH was added to the center well of the vessels to absorb the carbon dioxide. Changes in gas volume could be read to the nearest 0.1 $\mu$l. Because of differences in amount of oxygen consumed by different instars due to their weight differences, 15 second instar, 10 third instar, and 4 fourth instar larvae were used per vessel. First instar larvae and pupae were not used because of their rapid physiological changes. Each group of replicates in these experiments was continued for 24 hours. During this time the vessels were continuously shaken at a moderate rate. Readings were taken every two hours.

RESULTS AND DISCUSSION. The first series of experiments was performed to see whether there actually was an endogenous influence on respiration. In this series of five groups of replicates, four vessels in each group were assigned to each of the three instars tested. The runs were started at 10:30 a.m., 12:30 p.m., 4:30 p.m., 6:30 p.m., and 10:30 p.m. to avoid influencing the results by starting all experiments at the same time of day. These particular times were chosen in order to avoid giving the animals a light cue other than the last "dusk" and "dawn" they had experienced prior to removal from the insectary. All animals were thus removed during the light phase including those animals started at 10:30 p.m. There is a necessary time lag between the time of removal and initiation of the experiment due to preparative procedures and acclimation.

The effect of time spent in the vessels was analyzed for its influence on respiration (Southwood, 1966) by least squares regression. The individual raw data were then adjusted using the regression coefficient (Snedecor, 1956). Staggering the times at which the experiments were begun sufficiently canceled out the effects of fatigue, etc. that, after statistical adjustment, only the absolute values were slightly changed. Neither the shapes of the curves nor their relationships were modified. These data are presented on a per vessel basis since, at the time the experiments were performed, a suitably sensitive balance was not available to put them on a weight basis. The influence of weight and other factors on respiration will be discussed in a later paper. The data may be put on a per individual basis by dividing by the number of individuals per vessel as given above.

The results of this series of experiments are graphed as the lower three curves in Figure 1. All curves display significant fluctuations ($p<.05$) using Duncan's New Multiple Range Test except that for the third instar, which has a larger variance. Nevertheless, the curve for third instar shows highly significant ($p<.0001$) correlations, using Kendall's tau, of .970 and .536 with fourth and second instars respectively. Metabolism is minimal just before "dawn," after which activity increases to a peak in midmorning and declines to a plateau in the afternoon. This pattern is understandable if we assume that optimal environmental temperatures occur in midmorning, and that decreased activity occurs at times when temperature is above or below this optimum. As environmental temperatures cool into the optimal range in late afternoon, there is again an increase in activity that lasts until midnight, followed by a decline as environmental temperatures cool below the optimum. Although these animals had been kept under conditions of constant temperature for their entire life, these endogenous changes in respiration appear to be correlated with

changes in temperature under natural conditions. This type of correspondence is apparently not unusual (Ashoff, 1966).

From the above data it appeared that even though the animals were treated under constant light, the time of their accustomed "dawn" strongly influenced the pattern of fluctuations. In order to test whether light was in fact the entraining agent, an additional series of five replicates was run. Fourth instar larvae were used that had been exposed to light during what would normally have been the dark portion of their previous diel cycle. The times of day that these runs were initiated are the same as for the first series. Thus animals which were begun at 10:30 a.m. had not been exposed to darkness since 7:00 a.m. the previous day. These results are plotted as the upper curve in Figure 1.

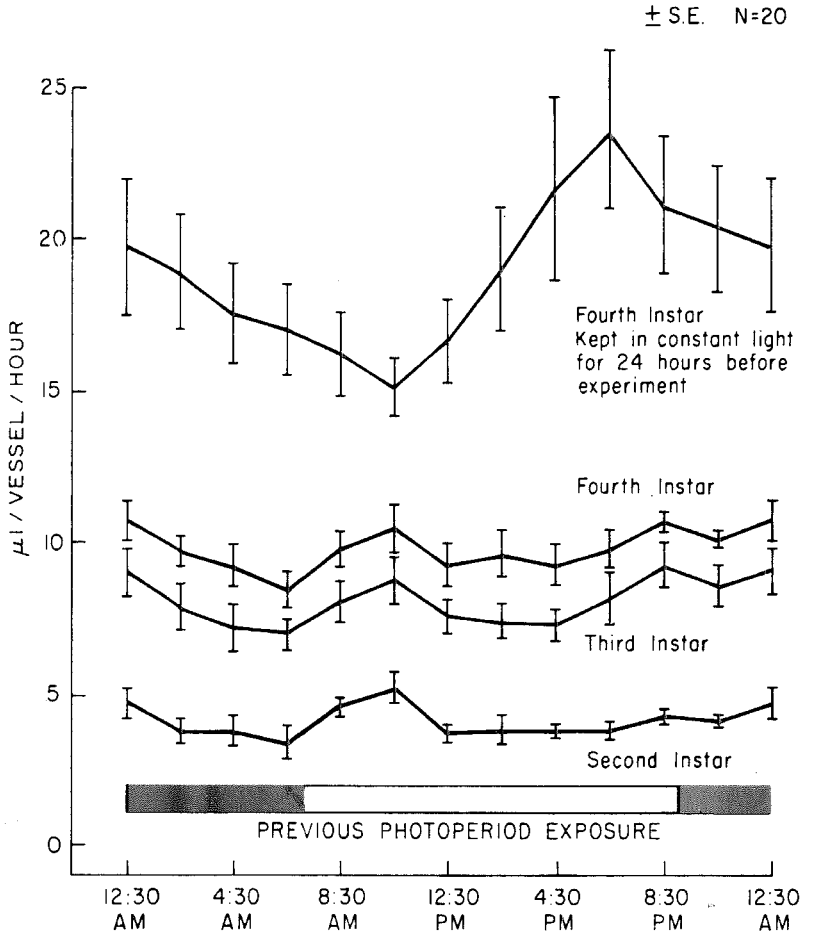


FIG. 1.—Fluctuations in the respiratory pattern over a 24 hour period. Lower three curves represent animals which were started during the light portion of their normal diel cycle (indicated by the bottom bar). The upper curve represents fourth instar larvae kept in constant light during the entire day prior to measurement.

Respiration was increased by approximately a factor of two. We may surmise that continual exposure to light was a stress condition to which the animals responded with increased activity due to negative phototaxis. Instead of two peaks as in the first series of experiments, there is only one. Ashoff (1966) has noted that under conditions of constant light, the second peak will sometimes disappear. In this experiment it is not clear which of the two peaks has been eliminated or whether the two peaks have fused, since the remaining peak lies midway between the two former peaks. Since the pattern of response is extensively altered when the normal diel cycle is interrupted by a period of continued light, light would appear to act as the entraining agent for the endogenous diurnal respiratory pattern.

SUMMARY AND CONCLUSIONS. From these experiments it appears that respiration of second, third, and fourth instar larvae of *Culex pipiens* is similarly modified by endogenous influences. This endogenous pattern of respiration is probably adaptive in response to temperature fluctuations in the environment. The timing of this pattern depends on light cues for entrainment.

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