INTERRUPTION OF DIAPAUSE AND REARING LARVAE OF *CULISETA MELANURA* (COQ.) [1]

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Culiseta melanura is a mosquito species that has been found in most of the states east of the Mississippi River. The species breeds primarily in heavily shaded, permanent fresh water swamps and marshes. The larvae generally develop in holes beneath tree roots or stumps, under rock ledges, and beneath root systems of aquatic plants. It has long been known that the larval stages can be found throughout the year, and both Smith (1904) and Dyar (1905) quote unpublished notes of Mr. J. T. Brakeley indicating that the larvae could be collected in May from water in places that had been frozen over during the previous winter. Generally similar life histories, with an overwintering larval phase, have been observed for the species in studies of its biology in New Jersey (Burbutis and Lake, 1956), Massachusetts (Hayes, 1961), Georgia (Silverly and Schoof, 1962), and Alabama (Stamm, Chamberlain, and Sudia, 1962).

Burbutis and Lake (1956) noticed that the cessation of larval development, or diapause, during the winter months in New Jersey did not appear to be a consistent or obligatory characteristic. They reported that a group of larvae brought into their laboratory in the winter of one year did not pupate until the following spring; whereas, those brought in the following winter continued normal development and progressed to the adult stage in a relatively short time. Larvae collected during November and December in Georgia required up to two months to develop from third or fourth instar to adults (Love and Goodwin, 1961), and development time was not affected by variations in the length of the photoperiod. Wallis (1962) found that he could stimulate pupation of significant numbers of larvae by adding a liver concentrate to their dietary regimen.

The purposes of this paper are to report the success attained in terminating larval diapause of *C. melanura* by increasing the length of the photoperiod in addition to initiation of larval feeding, and to report the results obtained in studies of rearing *C. melanura* larvae on various diet regimens.

MATERIALS AND METHODS. The *C. melanura* larvae utilized in the diapause studies were collected during late autumn from natural breeding sites in swamps near Taunton, Massachusetts. These third and fourth instar larvae were brought to the laboratory and transferred to 2-gallon capacity glass aquaria. Water from the swamp habitats in which the larvae were collected was used to fill the aquaria. The aquaria were stored in a household refrigerator that was maintained at 5±2° C. Several thousand larvae were maintained in each aquarium. No larval food was provided to the refrigerated larvae; the water surface was skimmed occasionally to prevent formation of a pellicle on the surface. Throughout the winter months, as larvae were needed for various studies, they were removed from the refrigerator.

In our laboratory, tap water was found to be suitable for replenishing water lost

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by evaporation from the storage aquaria and for rearing larvae. The water source was deep wells located on the grounds of the Paul A. Dever State School in Taunton, Massachusetts. The water usually was not chlorinated, but an arrangement had been made to advise us prior to periodic chlorine treatments. Such treatments were infrequent, and distilled water was used for larva rearing for 2 or 3 days following such treatments.

Initial tests on the effect of combinations of food and prolonged photoperiod for interruption of diapause were conducted by placing 10 fourth instar larvae in 4-ounce jars filled with water. Larval food consisted of 2 cc. of a 5 percent solution of liver concentrate [4, 5] in distilled water and a pinch of brewer's yeast sufficient to cover the surface of the water.

The extended photoperiod of 20 hours was provided by two 200-watt incandescent lamps, each controlled by an individual automatic timer. No other lengths of extended photoperiods were tested. The maximum and minimum normal winter daylight periods during the period of larval diapause, November through March, ranged from 9 hours and 10 minutes to 12 hours and 41 minutes.

The room temperature during the studies was 25±3° C. The diapause and rearing studies conducted with larger numbers of larvae were done using 2 x 7 x 11 inch aluminum pans coated with beeswax.

RESULTS. Larvae collected after October were found to be in diapause. The preliminary trials with 10 larvae per jar indicated that an increase both in photoperiod and in feeding was needed to terminate diapause. Two trials were conducted in which the larvae were fed but the photoperiod was not increased, and another two trials in which the larvae were not fed and the photoperiod was increased to 20 hours. No pupation occurred among any of the larvae in these tests, whereas, 80 percent pupation occurred within 16 days among larvae that were in jars and fed and exposed to the 20-hour photoperiod.

Subsequent tests in which larger numbers of larvae were tested in enamel pans showed that about 70 percent pupation could be expected when the larvae in diapause were fed and exposed to the 20-hour photoperiod at room temperature. This method was established and routinely used for interrupting larval diapause.

The swamp habitats for the larvae of *C. melanura* were found to have a hydrogen-ion concentration range from pH 5.0 to 6.0. Tests of the larval rearing medium showed that the liver-yeast larval rearing medium pH was 5.0 at the time the pans were initially set up. However, as the laboratory cultures aged, the pH increased to 7.0, the cultures often became cloudy, and formed heavy surface pellicles. The substitution of ground, growing-type chicken feed was found to eliminate the detrimental effects of the liver-yeast regimen. The chicken feed type of rearing medium remained slightly acid, clear, and did not form excessive amounts of scum.

Ninety-six percent pupation in 3 weeks was obtained in four tests with 100 first and/or second instar larvae reared in enamel pans in 1.5 liters of water and fed 0.12 g. of the ground chicken feed Monday through Friday. Three other chicken feed regimens were tested under similar conditions and yielded 67 to 82 percent pupation in 3 weeks (Table 1).

Summer trials also were conducted with first instar larvae from eggs that hatched

TABLE 1.—Results of laboratory rearing first and/or second instar larvae of *Culiseta melanura* with ground, growing-type chicken feed.

Feed regimen grams	days/week	Number of trials [1]	Percent pupation 2 weeks	3 weeks
0.12	5	4	88	96
0.24	5	8	36	76
0.12	3	4	16	67
0.24	3	4	44	82

[1] One hundred larvae per trial.

[4] Wilson Laboratories, 4221 S. Western Avenue Boulevard, Chicago, Illinois.

[5] The use of trade names and commercial sources is for identification purposes only and does not constitute endorsement by the Public Health Service or by the U. S. Department of Health, Education, and Welfare.

in the laboratory. These larvae were hatched from eggs laid in the laboratory by mosquitoes collected in swamps, and from eggs from rafts collected in the swamp and brought to the laboratory. Mosquitoes, fed on baby chicks, laid eggs in rafts the third or fourth day after the blood meal, and the eggs usually hatched the second day after being laid. From a total of 36 egg rafts, 5537 larvae were hatched and were counted when they became second instar larvae. Thus, the average number observed was 153.8 larvae per egg raft.

The newly hatched larvae were fed ground chicken feed, and four different feeding schedules were tested. Ninety-five percent pupation was obtained in 11 trials with the regimen of 0.12 g. fed daily, Monday through Friday, to the larvae from a single egg raft in 1.5 liters of water. A 93 percent pupation was obtained in 4 trials with 0.12 g. of feed on Monday, Wednesday and Friday, and 92 percent pupation was obtained by feeding 0.24 g. of the 5-day week schedule. Only 39 percent pupation occurred in 9 trials when 0.36 g. was fed 5 days per week. Prior to the feeding of the larvae, the water surface of the larval rearing medium was skimmed with a strip of paper toweling to remove any pellicle that had formed. In these trials about 4 weeks elapsed from the time the larvae hatched until they emerged as adults. The data obtained in rearing larvae from eggs of *C. melanura* are given in Table 2.

TABLE 2.—Results of laboratory rearing newly emerged larvae of *Culiseta melanura* with ground growing-type chicken feed.

Feed regimen grams	days/week	Number of trials[1]	Percent pupation[2]
0.12	3	4	93
0.12	5	11	95
0.24	5	15	92
0.36	5	9	39

[1] Each trial used the larvae that hatched from a single egg raft.

[2] Pupation occurred within 32 days from the day of hatching.

DISCUSSION. Wigglesworth (1947) noted that diapause, or the arrest of development, is to be regarded as a biological means of surviving adverse conditions, notably the winter cold. In the United States *Anopheles barberi, Aedes triseriatus, Culex erythrothorax, Culiseta inornata, C. melanura, Mansonia perturbans, Orthopodomyia signifera* and *Wyeomyia smithi* are species that are known to be capable of overwintering as larvae, and several of these species have been the subject of studies to determine the factors or conditions necessary for termination of the diapause. Baker (1935) concluded that increased length of photoperiod, from about 9 hours to about 16 hours, would terminate diapause in New York larvae of *A. barberi* and *O. signifera*. In his report he indicated that he also fed the larvae powdered leaf meal and lettuce. In studies of *A. triseriatus* in Georgia by Love and Whelchel (1955) it was shown that extended photoperiods at water temperature of approximately 29° C stimulated pupation of larvae in diapause. Chapman (1959) reported that the overwintering of the larvae of *Culex erythrothorax* in Nevada was principally contingent upon temperature, and that larvae changed instars, pupated, and emerged as adults when maintained at room temperature. In Connecticut, Wallis (1962) reported that feeding 5 percent liver concentrate solutions stimulated pupation among approximately half of the overwintering *C. melanura* fed in some trials, and he found essentially the same results upon retreatment of the surviving larvae after a 4-week rest period. Wallis did not indicate the effect of increased photoperiod.

We found that feeding the larvae and extending the photoperiod to 20 hours terminated the *C. melanura* larval diapause among 80 percent, or more, of the larvae treated. The technique was used successfully for two years for the routine production of adult *C. melanura* in the winter months during attempts to colonize the species.

The successful rearing of *C. melanura*

larvae in the laboratory was noted by Silverly and Schoof (1962) to be a prerequisite to needed studies on *C. melanura* biology. They encountered some difficulties with *C. melanura* larva rearing in Georgia that were not encountered in our studies or by Wallis (loc. cit.); e.g., rearing in enamel pans resulted in high mortality, and rearing in tap water was unsuccessful. In our studies *C. melanura* adults were readily reared from eggs hatched in the laboratory by feeding the larvae a regimen of ground, growing-type chicken food five times per week. The adults produced were robust and active, they fed readily on restrained chicks, and laid eggs in colony cages. Some fertile egg rafts were obtained, but the percentage of fertile eggs was not sufficient to sustain a laboratory colony.

SUMMARY. Development was reinitiated among larvae of *Culiseta melanura* in winter diapause by simultaneously increasing the photoperiod to 20 hours and providing a ground growing-type chicken feed. The laboratory technique was used successfully two years for the routine production of adult *C. melanura* during winter months in Massachusetts. Several dietary regimens were tested for rearing larvae from individual *C. melanura* egg rafts hatched in the laboratory. Ninety-two to 95 percent pupation occurred using diets of 0.12 g. three days per week, 0.12 g. five days per week, and 0.24 g. five days per week.

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