antibiotics in rearing larvae of species requiring algae as food.

8. Larvae reared in pans containing antibiotics were significantly larger than those in the control pans; most showed a size increase of 10–17 percent.

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THE EFFECT OF TEMPERATURE ON HATCHING OF EGGS OF THE MOSQUITO, *CULEX PIPIENS QUINQUEFASCIATUS* SAY.[1]

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INTRODUCTION. According to Bates (1949), culicine mosquito eggs laid in rafts normally hatch as soon as the embryo is fully developed. Unlike the eggs of *Aedes* species there is no special stimulus required for hatching, and it has been generally understood that development of the embryo and hatching are a direct function of temperature. It appears that Kirkpatrick (1925) and Boissezon (1930) are the only workers who have reported on studies of the duration of the egg stage of *Culex pipiens*. Kirkpatrick (1925) found that eggs of the Egyptian form of the species would hatch at 10.5° C. with an incubation period of 216 hours. He further reported hatching at the following temperatures and respective incubation periods: 13° C., 144 hours; 18.5° C., 72 hours; 21° C., 36 hours; 28° C., 32 hours; 30.5° C., 26 hours; and 34° C., 21 hours. Embryos survived 34° C. but were killed at 35° C. Boissezon (1930) obtained essentially the same results with one of the French forms when he found at 10° C. incubation required 216–264 hours, at 15° C. incubation required 48–72 hours, and at 20°–25° C. about 24 hours were required.

The main objective of this study was to determine whether the hatching time of the eggs of the mosquito, *Culex pipiens quinquefasciatus* Say is directly related to the temperature of the water upon which they are placed.

MATERIALS AND METHODS. A stock of *Culex pipiens quinquefasciatus* Say was established from five egg rafts from a colony which had been maintained in the Department of Entomology, University of Maryland for about 7 months. Mosquitoes in this colony descended from the colony at the Walter Reed Army Institute of Research which has been identified as the Malayan strain. The stock colony was reared in a cage heated with a Westinghouse heating pad (Model WP-29) controlled by a hermetically sealed Chromalox thermostat (Model WR-66). The cage measured 18 x 24 x 31 inches. The humidity was maintained at a fairly constant level by placing an enamel pan measuring 7½ x 12 x 2 inches containing five cellu-

[1] Condensation of a thesis submitted by the first author to the Faculty of the Graduate School of the University of Maryland in partial fulfillment of the requirements for the degree of Master of Science in 1963. Scientific Article No. A1106, Contribution No. 3548 of the Maryland Agricultural Experiment Station, Department of Entomology.

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lose sponges, each measuring 4 x 6 x 1 inches, in the cage upon the heating pad. The sponges were kept saturated with water. This arrangement afforded a very satisfactory method of maintaining a temperature of 80° F. ± 2° and a relative humidity of 78% ± 2%.

A separate emergence cage was used to eliminate open water in the stock colony cage. The gravid females were given water on which to oviposit only when eggs were required for study. Larvae were reared in enamel pans containing hay infusion and were fed two or three Purina guinea pig chow pellets per pan per day. As the adults emerged in the emergence cage they were collected daily with a modified battery-operated vacuum cleaner and were placed in the stock colony cage.

The food and water requirements for the adults were satisfied with water impregnated cotton wicks and sliced fresh or frozen apples. Females in the stock colony were allowed to feed on two young chicks once or twice a week. The young chickens were placed in harnesses similar to those described by Trembley (1955), and were allowed to remain in the stock colony cage overnight. At such times, it was estimated that the average number of females in the colony was 3,500.

As was stated previously, these blood-fed females were confined in the stock cage in the absence of any open water upon which to oviposit until such time as eggs were required for tests. When eggs were needed for the experiments, a stender dish containing hay infusion was placed in the stock cage. It was found through experimentation that oviposition occurred much more readily in darkness. This necessitated covering the cage with a black cloth.

Since this entire work was based on the timing of egg hatching at various temperatures, the age of the eggs had to be known at the time of the initiation of the particular test. This was accomplished by placing the oviposition water or hay infusion in the darkened stock colony cage for a prescribed time. It was found through experimentation that a minimum of 1 hour was required to obtain what were considered complete egg rafts. When a shorter period was used many incomplete rafts were formed.

A minimum period of 8 days was allowed after a blood meal prior to the placement of the oviposition water in the stock colony cage. It was found that this length of time was required to obtain a sufficient complement of eggs in the allotted 1-hour interval. The minimum time for oviposition was 4 days after a blood meal.

To create a check for the viability of each egg raft obtained, the raft was broken in half with approximately equal numbers of eggs in each half. This was accomplished with the use of a blood lancet, so that eggs near the separation line were not damaged. The total number of eggs in each half was then counted. These totals were then recorded and the particular egg raft segment was then ready for testing.

The testing apparatus consisted of a standard 4 quart battery jar fitted with a 1 pint mason jar supporting a 140 mm., 7-holed, desiccator plate as shown in Figure 1. The source of heat was a Model OA, Metaframe 25 watt, thermostatic aquarium heater. The 7-holed desiccator plate allowed for convenient placement of the heater tube.

For those tests which involved temperatures above the normal room temperatures the heaters were calibrated for the appropriate temperature and the jars placed on a table in the laboratory. In those tests involving temperatures below room temperatures the same apparatus was used except that the entire unit was placed in a small refrigerator. Temperature variations appeared never to exceed 2 degrees in either direction for either location. The interior space of the refrigerator maintained a constant temperature of approximately 40° F.

Containers to hold the egg raft in the battery jar bath consisted of 50 ml. beakers. These beakers were filled with distilled water after which the egg raft portion was placed on the surface. Tap water was used

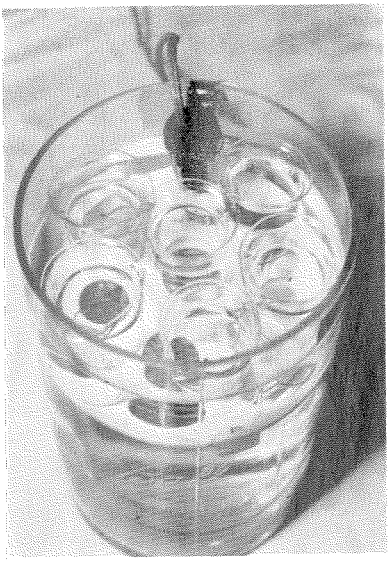

FIG. 1.—Apparatus for observing the hatching of *Culex* eggs.

as the bath water in the battery jar. The level of the water in the jar was then brought up to the same level as that of the distilled water in the beakers. It was found that seven 50 ml. beakers could be accommodated in the constant temperature tank simultaneously.

Only three of these constant temperature tanks were used at any one time. This allowed the maximum of 21 beakers containing egg rafts to be subjected to any of three different temperatures, during the same interval.

The temperatures used for these tests ranged between 45° F. and 100° F. with the majority of tests being conducted with 65°, 75°, 85°, and 95° F. A minimum of 1000 eggs was subjected to each of these temperatures.

Observations of hatching were made at 2-hour intervals, and the total number of larvae which had emerged during that time was recorded. When the presence of larvae within the beaker was noted at a particular interval the entire raft containing the unhatched as well as the hatched eggs was removed. This raft was then transferred into another beaker containing distilled water which had been pre-heated or cooled to the appropriate test temperature. This allowed those unhatched eggs to continue to hatch in an identical water temperature to which they had previously been exposed. It also prevented the raft from being exposed to drying influences of the air for any extended period. The first beaker now contained only the larvae which had emerged during the prescribed 2-hour interval.

The entire contents of this beaker were then transferred into a 10 cm. petri dish. The beaker was thoroughly washed with distilled water from a wash bottle to insure the removal of all larvae. The petri dish was then placed on an ordinary electric hot plate and heated to approximately 118° F. It was found that excessive heating of the water caused a considerable distortion of the larvae, and in some cases almost complete disintegration occurred. After all the larvae were dead the contents of the petri dish were poured through a filter paper inserted in a small Pyrex funnel. This technique of killing the larvae produced excellent results in that an accurate count of the larvae was possible. The larvae were well dispersed on the filter paper which was then flattened out and placed on the bottom of a 12.5 cm. petri dish marked with a pie-shaped grid to facilitate counting the larvae. This dish containing the filter paper was placed in the field of a low power stereoscopic microscope equipped with substage lighting. The actual counting of the larvae then proceeded, and after this the paper was allowed to dry. Even after drying, the larvae were well attached to the fibers of the filter paper, and the papers could be taped to a card and preserved as a permanent record.

RESULTS AND DISCUSSION. Tests were conducted to determine the maximum and minimum temperatures at which *Culex pipiens quinquefasciatus* Say eggs would hatch. Of a total of over 1,400 eggs subjected to 100° F. none was observed to hatch within 168 hours. Also, of a total

of 568 eggs tested at 45°, 50°, and 60° F. none was observed to hatch within 168 hours.

The response of eggs to four temperatures is given in Table 1. It can be seen

TABLE 1.—Hatching response of eggs of *Culex pipiens quinquefasciatus* Say at four temperatures.

Degrees F.	65	75	85	95
No. parts of egg masses	16	15	15	18
No. of eggs	1079	1318	1031	1243
No. hatched	600	923	736	530
Percent hatched	55.6	70.0	71.4	42.6
Avg. no. hours hatching time	68.4	32.9	29.0	26.6
Range hours hatching time	64–70	28–36	28–32	25–28

that the hatching time decreased in each test as the temperature was increased by 10° F. At 65° F. the percent hatch was 55.6 compared with 42.6 percent at 95° F. There was no noticeable difference between the percent hatch at 75° F. and 85° F., and the average hatching time differed by less than 4 degrees. At this range the hatching time was optimum. It is important to note that at 75° F. the hatching time was approximately 35 hours less or 52 percent less than that for eggs at 65° F.

Records of cumulative hatch at 2-hour intervals revealed that there was a narrow time latitude at each temperature. After hatching began it proceeded fairly rapidly.

These experiments show that water temperature is the only environmental factor which influences hatching of *Culex p. quinquefasciatus* eggs. There was no apparent difference in hatch of eggs placed on distilled water and hatch of eggs floating on a hay infusion where nutrients were available and from which gases were escaping. It is well known that this species of mosquito oviposits in polluted water. We observed that females when given a choice between hay infusion and distilled water usually oviposited on the former.

CONCLUSIONS. The following conclusions about the Malayan strain of *Culex pipiens quinquefasciatus* Say are suggested by this study:

1. At 95° F. embryonic development occurs rapidly and is completed in less than 27 hours, but mortality exceeds 57 percent.

2. At 65° F. embryonic development proceeds rather slowly and is completed in 68 hours, but mortality exceeds 44 percent.

3. The optimum temperature range for embryonic development is 75° F. to 85° F.; about 70 percent of the eggs hatch in about 30 hours.

4. Embryonic development stops at 100° F. and at 60° F.

5. Egg diapause is non-existent.

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