A SIMPLIFIED METHOD FOR LARGE-SCALE LABORATORY REARING OF *AEDES AEGYPTI* (L.) [1]

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Large numbers of mosquitoes are frequently required for such laboratory studies as basic physiological reactions, insecticidal resistance, and evaluation of insecticides and repellents. At our laboratory, large numbers of *Aedes aegypti* (L.) have been used in the preparation of extracts for use in studying reactions to mosquito bites. The rearing of mosquitoes for this purpose has required, in the past, many hours of tedious effort at the expense of the main problem under investigation. Recently, however, a method for mass-rearing *A. aegypti* has been devised, with the result that the time spent in rearing has been reduced to a fraction of that required previously.

Methods in general use for rearing *A. aegypti* under laboratory conditions are fairly standard, and, except for minor variations, follow the procedures described by Granett and Haynes (1944), and Trembley (1944, 1955). Large-scale rearing by these procedures involves the use of a large number of trays. The handling of so many trays each day requires careful manipulation, and is, therefore, time-consuming. The separation of pupae from larvae is likewise a tedious and slow process.

DESCRIPTION OF REARING UNIT. In an attempt to reduce the time spent in rearing, a unit was constructed in such a way that large numbers of larvae could be reared to adulthood without separating pupae from larvae while at the same time eleminating the use of a large number of trays. Figure 1 is a photograph of the unit, and Figure 2 is a sectional view. The tank was 30 in. long, 14 in. wide, and 36 in. high, and was made of galvanized iron. The top section was tapered, beginning 24 in. from the bottom, to form an opening 6 in. square. Three

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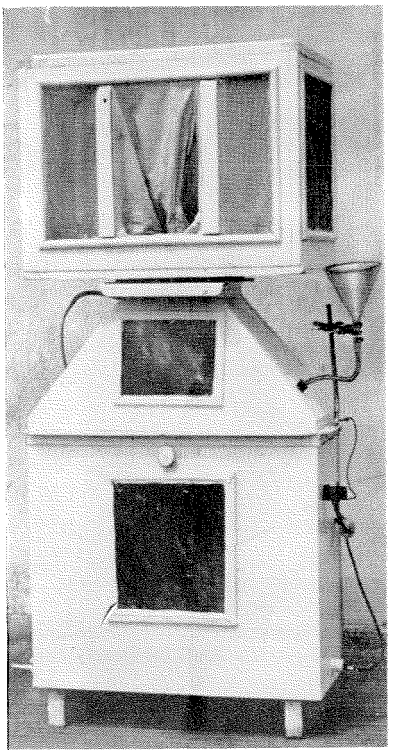

FIG. 1. Photograph of rearing unit.

pieces of metal screening (f) were soldered within the tapered portion to provide resting places for newly emerged adults. A slide (d) was fitted across the opening at the top of the tank. Sponge-rubber stripping was cemented to the top edge of the tank to form a seal between the tank and a removable cage (a).

The cage measured 30 in. long, 20 in. wide, and 20 in. high, and had in its floor a centrally located opening of 5 in. diameter. The opening could be closed by a swing-type cover (c). During the time that the cage was being stocked with mosquitoes, a removable metal-screen funnel (b) was placed over the opening to deter mosquitoes from re-entering the tank.

Tap water was added to the tank to the 24-inch level (approximately 165 l.). A copper air-line (h) was inserted, and its position was adjusted to about 4 in. below the surface of the water. The air-line had three orifices, .015, .078 and .094 in., the largest being near the closed end of the copper tube. Aeration at 600 ml. per hr. was used to inhibit film formation on the surface of the rearing medium. The amount of surface disturbance was regulated by minor adjustments in the position of the air-line and in the rate of aeration. The room was maintained at 26° to 28° C. The rearing medium was held at 28° ± 0.1° C. by means of a 1000-watt heater (j) and a thermoregulator (g) built into the tank. A funnel (e) was used to add food and larvae to the rearing medium. A tap (k) enabled the tank to be drained as necessary. Two windows were provided in the front of the tank to permit inspection of conditions inside the tank (see Fig. 1). Normally these windows were kept covered with black cardboard.

REARING METHOD. Filter papers on which *Aedes aegypti* eggs had been deposited were immersed in water containing a few drops of yeast suspension and left overnight for the eggs to hatch. The following day the papers were removed, the number of larvae was estimated, and the water containing the larvae was poured into the tank. Estimates were made by counting 1,000 larvae into a minimum amount of water in a test tube and using this tube as a standard for making up additional lots of 1,000 larvae. Dog biscuits and Fleischmann's dry yeast were soaked in water and added to the contents of the tank. The feeding schedule was as follows:

Days after Hatch	Dog biscuits (mg. per larva)	Yeast (mg. per larva)
1	1	0.2
5	2	0.2
7	2	—

Pupation began on the sixth day after hatching. Emergence of adults began on the eight day, reached a peak on the tenth to twelfth day, and was 99 percent complete on the sixteenth day. When the cage

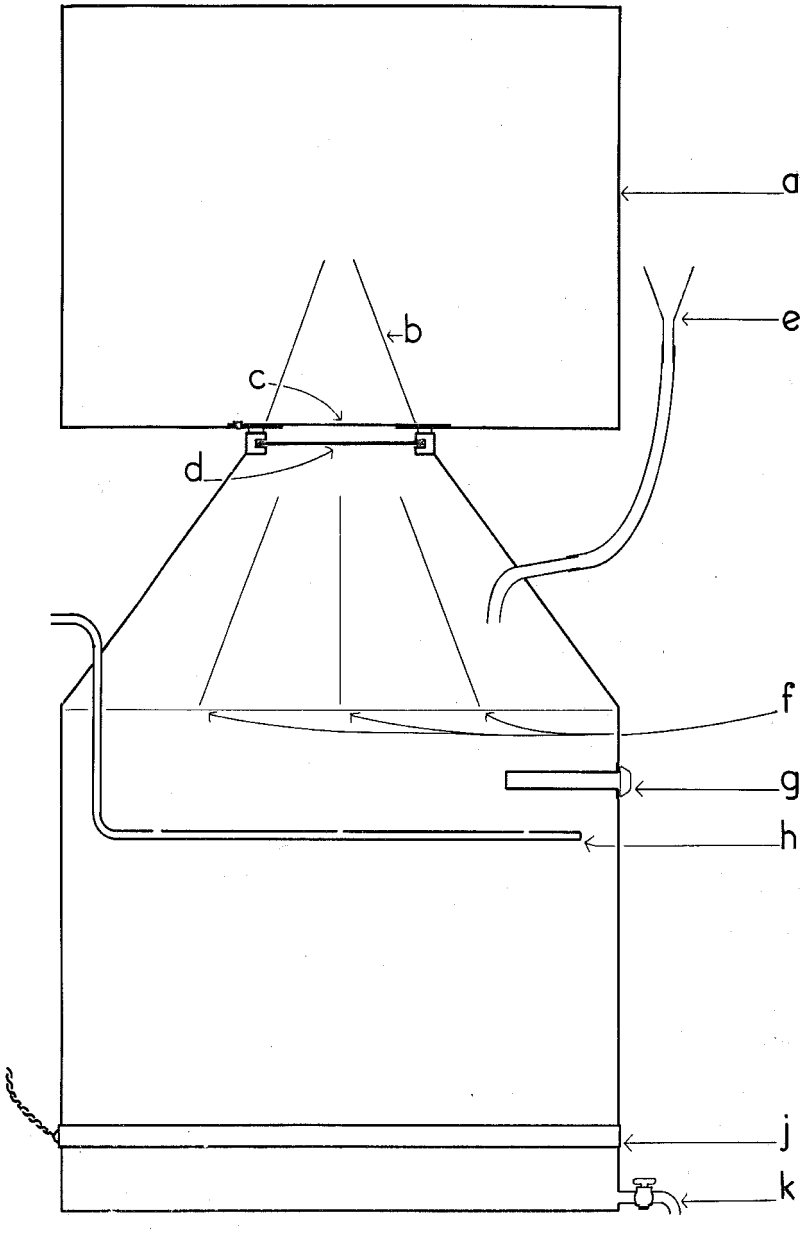

Fig. 2. Diagrammatic sectional view of rearing unit.

was fully stocked (about 4,000 mosquitoes as based on past experience with this size of cage), the slides (c) and (d) were closed, the screen funnel (b) was removed, and the full cage was replaced by an empty one. The slides were opened, the screen funnel was inserted, and the new cage was ready to be stocked.

Several trials were made with three rearing units to arrive at the feeding schedule described above and to obtain an approximation of the maximum number of larvae that could be reared satisfactorily in one unit. As a result of these trials, it was found that 40,000 larvae per unit (about 4 larvae per ml.) could be reared at one time, and that this number produced large, healthy-appearing adults whose longevity did not differ noticeably from those reared by the tray method. Larger numbers than this usually resulted in undersized adults and in prolongation of the aquatic stages. The addition of amounts of food larger than those shown in the feeding schedule frequently was followed by the formation of a surface film in the corners of the tank. This suggests that a circular rather than a rectangular tank might be preferable. The dimensions of the tank were arbitrarily chosen, and no attempt was made to determine an optimum surface-to-volume ratio of the rearing medium. Although considerable numbers of larvae were found to be feeding at the bottom of the tank at any given time, this does not preclude the possibility that a larger number of larvae per ml. could be reared satisfactorily by altering the surface-to-volume ratio, thus effecting a saving in the space requirements. Examination of the sediment in the tank at the end of satisfactory runs indicated that the mortality of the aquatic stages was negligible. Also, counts made of the adults obtained from such runs were within 20 percent of the estimated number of larvae added to the tanks. The rate of production by the above method is, therefore, approximately 2,500 adult mosquitoes per rearing unit per day. The time required to maintain this rate of production in three units is about two hours per week per unit. Although these units have been used to rear only *A. aegypti,* it seems likely that the method could also be used to rear *A. atropalpus* (Coq.) and probably some of the other mosquito species.

After each of the runs referred to above, the three rearing tanks were drained and washed out thoroughly. To determine whether it is necessary to discard the rearing medium after each run, a fourth unit was kept continuously stocked with *A. aegypti* larvae. The population of larvae in the tank varied from time to time, but never fell below 5,000. The periodic requirements for additions of yeast and dog food were estimated by visual inspection of the contents of the tank. This unit was operated continuously for eight months, and at no time was there any evidence that products toxic to the larvae had accumulated. The tendency towards film formation from addition of excess food appeared to be much less than in a tank recently placed in operation. On the few occasions that a film began to form on the medium, the film was removed with a bottle brush, and about one-quarter of the medium was drawn off and replaced with tap water. This method was found to be adequate for preventing further film formation.

The fact that the same larval medium can be used continuously means that a great saving of distilled or treated water is possible in areas where tap water is not suitable for rearing mosquitoes. The continuous method is especially useful during periods when the colony is merely being maintained. For maximum production, however, a chemical method for determining the amount of food to be added periodically to the medium would be desirable.

SUMMARY. A method is described for rearing large numbers of *Aedes aegypti* with little effort and attention. The rearing unit consists of a collecting cage

mounted on top of a thermoregulated tank. Aeration is used to inhibit film formation on the surface of the rearing medium. When the collecting cage becomes filled with mosquitoes, it is easily replaced by an empty cage. One of these units has been used to rear *A. aegypti* continuously for eight months with no signs of accumulating toxic products in the rearing medium.

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