REARING INDIVIDUALLY ISOLATED MOSQUITO LARVAE[1]

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There are many occasions which call for a simple, practical method of rearing large numbers of isolated, individual mosquito larvae for experimental purposes. A relatively simple device for isolating larvae for study purposes has been developed in this laboratory within the past few months. This device, known as a "float," makes possible the handling of a large number of larvae and eliminates many problems usually encountered.

The principal part of the float is a piece of Plexiglas, five by ten inches in size, and ¾ of an inch thick. This plastic, an acrylic polymer, is a product of the Rohm and Haas Company. Holes to be used for rearing chambers were bored through the plastic using a power drill with a ½ inch bit. The holes were spaced ½ inch apart, and arranged in five rows; each row has ten holes for a total of fifty in the piece of Plexiglas.

A piece of white nylon hose, 51 gauge, 30 denier, such as are worn by nurses and hospital attendants, was attached over one surface of the plastic. This closed one end of each of the holes which were then used as rearing chambers. It was found that the mesh was small enough to prevent the escape of first instar larvae, but large enough to allow free circulation of water and certain types of food material.

Duco cement, diluted with ethylene dichloride, was used in affixing the cloth to the plastic block. A small camel's hair brush was used in applying this adhesive. The cement was applied around each hole to insure that the separation of the chambers was complete, and to prevent larvae from being trapped between the cloth and the plastic.

In use, the float is suspended in an enamel pan with the side covered by the piece of hose on the under surface. Any of several methods of suspension may be used, and these will be mentioned shortly. Water is maintained in the container at a level where each compartment will be partially flooded. One larva is placed in each compartment which serves as a rearing chamber. Each compartment is numbered, and individual records are kept on the ecdyses of each larva.

When pupae appear, a small vial is inverted over each chamber and the adults are trapped as they emerge. Screw-top vials of the one dram size fit well in the openings and are used for this purpose. A small strip of filter paper is placed in each vial to allow for a better resting surface for the emerging adults. Adults have been left in these vials for as long as two days without any apparent harm. The ease with which the adults can be handled in this way is an important factor.

It has been mentioned that the float can be suspended in the container in a number of different ways. In our first attempt, two empty bottles with screw-tops were attached to the block in such a way as to give it sufficient buoyancy to remain at the surface of the water. This required a large container of water, and the float, being movable, was awkward to operate. Later, small holes were bored in the corners of the piece of plastic and short pieces of glass tubing, approximately three inches long, were inserted in each. These rods serve as legs upon which the block of plastic is supported (Fig. 1). The water level is kept constant by using an inverted flask containing water as a reservoir. A tube runs from the flask to the surface of the water in the pan. When the water level falls below the end of the tube, air passes up the tube into the flask; this permits water to run into the pan replacing that which has evaporated. This method has proved to be satisfactory and it is the one now being employed.

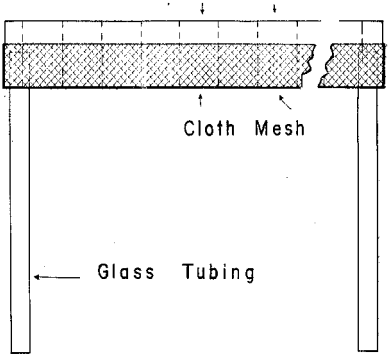

FIG. 1. Diagram of float, side view. The cloth mesh which covers the under surface is folded over and glued to the sides of the float to allow for better attachment.

The methods used in supplying food to the isolated larvae have varied somewhat. If food materials such as *Paramecium*, powdered dried brewer's yeast, or Bates Medium S are used, they are

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added directly to the water, since they easily pass through the mesh of the nylon hose. When larger particulate food, such as ground-up dog food, is used, it is added individually to each compartment. Detritus and wastes are removed daily from the chambers with a medicine dropper. Any foreign material or scum that collects on the surface of the water within the individual chambers is removed by drawing small squares of filter paper over the water's surface.

It has been found that there is less mortality among the larvae, both in mass and individual rearing, if the medium in which the larvae are reared is slowly circulated. This is provided for by compressed air; the air passes into a plastic tube and through an air stone into the water. The air stone is of the type often used in aquaria and which serves to disperse the air so that a series of fine streams pass into the water, forming many small bubbles. The air flow is controlled with a small needle valve. The water in which the float is suspended is circulated in this manner. To prevent air from collecting along the under surface of the float, the air stone is placed at one end of the enamel pan, the float at the other.

The use of this float has many advantages. This apparatus is simple and requires only a small amount of space. A minimum amount of daily care is required and data are easily and speedily recorded. All of the materials used in the construction of this equipment are either plastic or glass and do not react chemically with any reagent normally encountered.

This equipment has been in use only for a few months and, as yet, has not been tested with a great variety of different kinds of mosquito larvae. Several species of *Culex* and one of *Culiseta* have been reared successfully in this fashion. The basic elements of this method are simple, however, and can easily be modified to fit different standards which experimentation may demand. With these reservations and comments, it is hoped that this technique can be further adapted for experimental purposes.