# A METHOD FOR MAKING A SURVEY OF FLOODWATER MOSQUITOES [1]

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Populations of floodwater mosquitoes and those of grasshoppers have so many characteristics in common that similar methods for forecasting them may be used. The egg is the only stage in which populations of both are concentrated and stable. Eggs are present for the greater part of the year in recognizable, concentrated sites, but later stages, and especially adults, disperse widely over feeding sites. Observers of grasshoppers have found that data on distribution of eggs are the most reliable for forecasting abundance. Means for examining vast areas of the prairies and plains of central U. S. for eggs of grasshoppers have been devised (Shotwell, 1935), and plans for combatting outbreaks on the basis of data obtained have been made for 2 decades. Supplementary surveys of nymphs and adults affect local changes in the general plan.

Data on populations of floodwater mosquitoes (*Psorophora* and *Aedes*), like those for grasshoppers, indicate that plans for abatement may be made from surveys of eggs, supplemented by observations on the distribution of larvae and adults. The problem is to devise a rapid, dependable procedure for finding and identifying the eggs. Eggs are restricted in their distribution to areas subject to transient inundation. They are further restricted in all but a few instances to portions of shaded areas bearing plant debris, such as lodged plants, masses of algae or leaf mold. Some eggs are on the soil below the plant debris; others are in the debris itself, and still others may be found plastered to the walls of cracks in the soil or holes made by crayfish. They are present throughout most of the year and are always present in fall, winter and early spring. Once sites are known, mechanics of obtaining samples bearing eggs are simple. Identi-

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fication, too, is rapid and dependable (Horsfall, *et al.,* 1952 and Horsfall and Craig, *in press*). Supplementary surveys of larvae and adults may be used for short term forecasts in abatement operations.

SAMPLING.—Surveys for eggs may be used for determination of general distribution or for comparison between sites as to productivity. In preliminary stages of a survey, the former is the objective and permits extensive coverage because few samples need be taken from any site. Detailed comparative data require more extensive sampling of each site. Samples should be taken from all zones representing sites for oviposition. Numerous contiguous or spaced samples should be taken along profiles extending from the line of maximum flood to that lowest in the depression.

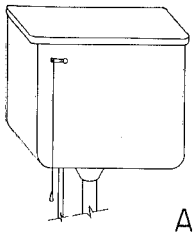
The equipment needed is simple and readily portable. Where samples are to be taken above the water table, the minimum equipment necessary is (1) a pointing trowel 4–5 inches long having sharp edges, (2) a board 6 inches square (¼ ft.$^2$), and (3) a supply of bags of plain brown paper or those called garbage bags. Samples from submerged sites may be taken with the following: (1) a pointing trowel, (2) a cutting square, and (3) a flat spade. The "cutting square" is much like a large cookie cutter. The cutting edge is a metal band forming a hollow square 6 inches on a side and closed at the top. For convenience a handle attached to the wooden face makes manipulation somewhat easier.

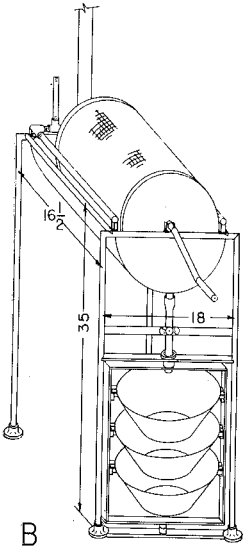
Samples suspected of bearing eggs may be obtained as follows: When dry samples are to be taken, the square is placed on the surface debris and outlined with a pointing trowel by cutting to a depth of about 1 inch into the soil. The sample is then cut loose from the soil below and placed in a paper bag properly marked for site and location. Sampling below water involves use of the "cutting square." The sharp edge of the cutter is put in place and forced into the soil. The spade or trowel is forced beneath the sample while the cutter is in place. The cutter, sample and spade are lifted out of the water together.

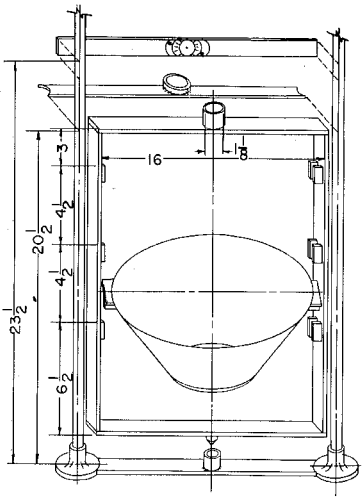
Separation of eggs takes place in the laboratory. Samples brought from the field are stored in a cold room or household refrigerator for holding. The act of separation involves 3 stages. Large debris is removed by washing and screening. Heavy material such as soil is separated by sedimentation. Eggs are collected from the last bit of debris by optical means.

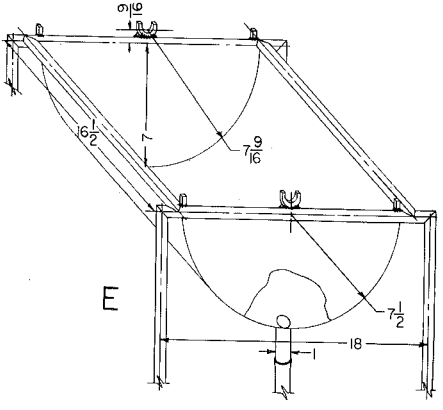
SCREENING.—The apparatus for breaking up a sample and removing most debris consists of 2 parts: (1) a water bath in which a set of 3 concentric cylindrical screens is partially immersed and (2) a set of 3 nested screens one above the other (Pl. I, fig. B). The cylindrical screens in the water bath are bolted together to form a unit as shown in Pl. 1, fig. F. The mesh of the cylinders decreases from inner to outer in the ratio of 4, 8 and 16 meshes to the inch. The whole set is closed at each end by metal caps perforated for a central shaft. The shaft of the cylinder fits into two bearings at each end of the vat (fig. E). The vat holds about 5 gallons of water obtained from a flush tank (fig. A). The nested or superimposed screens are shown in the lower part of fig. B and in detail in fig. D. Each screen forms the flat bottom of a truncated cone of galvanized metal. In order from top to bottom the screens have 40, 60 and 100 meshes to the inch. Each screen can be removed from a stand that can be turned in the manner of a revolving door. A large rubber tube with a pinch valve serves to drain the vat into the nested screens.

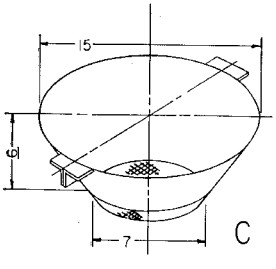
The operation of the device is as follows: Single samples are placed into the inner screen of the concentric set, the cylinder is closed, and the whole is placed in the vat. The vat is filled with water from the flush tank. By means of the crank the cylinder of screens is rotated at a rate of about 50 r.p.m. for a total of 125 revolutions. In practice, rotation is first 25 turns in one direction, then 25 turns in reverse until the sequence is complete. While completing the last 25 turns, the



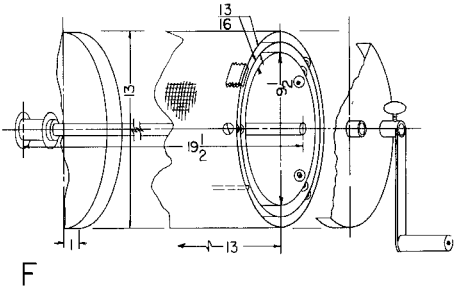




PLATE I. Illinois Mosquito Egg Separator

Fig. A. Flush tank
Fig. B. Assembled separator
Fig. C. Cone sieve
Fig. D. Cone sieve assembly
Fig. E. Detail of water bath
Fig. F. Detail of cylindrical screen assembly

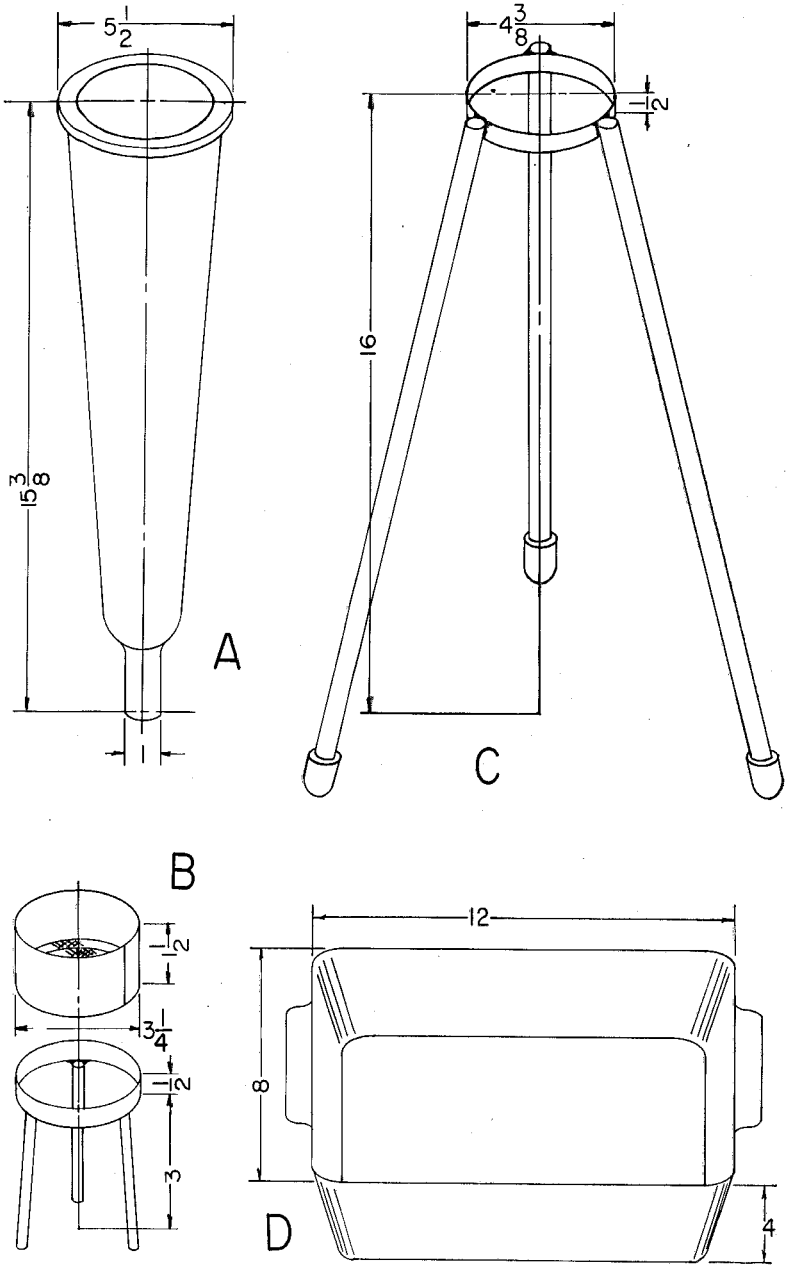



PLATE II. Apparatus for Flotation

Fig. A. Percolation funnel
Fig. B. Transfer screen assembly
Fig. C. Stand for percolation funnel
Fig. D. Plastic pan

vat is drained through the rubber port into the top screen below. As the vat is drained jets of water are sprayed over the turning cylinders. The jets come from a row of holes in a pipe along each side of the top of the vat as shown in Pl. I, fig. B. A treadle valve permits an operator to flush out the vat and keep both hands free. Debris collected on the various nested screens is subjected to a fan-shaped jet of water from a nozzle so as to wash eggs through to the bottom screen. Only silt and the finest debris pass through the bottom screen. All eggs are retained. Eggs and associated debris are washed from the bottom screen to a transfer screen (Pl. II, fig. B) for temporary storage.

FLOTATION.—Further separation of eggs from debris is based on differential densities and may be done in saturated solutions of technical NaCl in tap water. Equipment needed includes (1) percolation funnel (2-liter capacity) and stand (Pl. II, fig. A and C), (2) air pump, (3) casserole (150 ml. capacity), (4) plastic pans (Plate II, fig. D) and (5) wash bottle. An air pump such as is used in aeration of aquaria is suitable.

Flotation is accomplished in 2 stages. During the first the sample from the transfer screen is rinsed into a stoppered percolation funnel containing about 1½ liters of saturated salt solution. The solution is stirred by means of a long glass tube through which air is passed from the pump. After stirring for a minute or two, the bubbling-stirrer is removed and the contents of the funnel are allowed to stratify. The eggs together with a small amount of debris will float to the top while most debris will settle. A few minutes later heavier debris at the bottom is drained through the hole in the base (Pl. II, fig. A). Remaining material that is floating and trapped along the walls of the funnel contains the eggs. It is collected in a transfer screen.

The collection on the transfer screen is then rinsed into a casserole with tap water. At the option of the technician this collection may be treated to a second flotation directly or may be stored in a cold box at about 4° C. for an hour or overnight. Whichever course is followed the floating debris and most of the water are decanted as waste, and the residue is flooded with the saturated salt solution. Eggs and the small amount of debris will float as a layer on top of the solution. This layer is decanted into a transfer screen and rinsed into another casserole with tap water.

Optical separation is the final act and is carried out in 2 stages. During the first, eggs are removed from the last bits of debris, and during the last stage, they are separated according to species. For the first phase the operator needs a stereomicroscope with a wide field and a resolving power of about 10 diameters. Accessory equipment includes small casseroles, dropping pipets and a wash bottle. Residue from the final flotation is examined in a casserole containing about 30 ml. of water. Eggs or debris are removed by means of a dropping pipet. The cleaned eggs are now ready for identification. A stereomicroscope with a resolving power of about 100–130 diameters as well as a black, non-reflective, shallow container holding water are necessary. Depressions in a spot plate coated with black, plastic electricians' tape are suitable containers. Eggs may be separated as to species from a mass of some 50–200 eggs. Then the eggs may be transferred to a casserole for counting.

STORAGE.—Eggs may be kept for future use either in a preserved or in a fresh state. In the former instance a solution of 10 percent formalin is satisfactory. Live eggs may be kept on moist filter paper in a moist chamber at room temperature for short intervals or in a household refrigerator when storage for longer time is required. They may be stored in water for long periods if the water is kept well aerated.

EVALUATION.—The technique outlined above is a dependable means for separating eggs by experienced technicians in this laboratory. On one occasion such a technician placed 100 eggs in each of 5 samples known to be free of any eggs of the test species. The rate of recovery was

between 81 and 89 percent. Another series of 5 samples was populated with only 1 egg each. The single egg was recovered from four of the 5 samples.

SUMMARY.—A dependable means for making surveys of floodwater mosquitoes based on sampling for eggs has been devised. It consists essentially of collecting samples of surface debris, bringing them to the laboratory, screening them, floating the residue and finally identifying the eggs microscopically.

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