Were this species capable of transmitting disease, this feeding pattern would make this mosquito a most efficient vector.

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SEPARATION OF AEDINE EGGS FROM SOIL SAMPLE DEBRIS USING HYDROGEN PEROXIDE

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The collection and analysis of soil samples from areas subject to transient inundation is a survey technique important to mosquito abatement districts where floodwater mosquitoes are a major problem. Samples collected in late autumn are of value in determining intensity and distribution of the succeeding year's spring generation. Summer collections are useful in determining site distribution of eggs prior and subsequent to inundation.

The Illinois Egg Separation Procedure described by Horsfall (1956) is adequate for separating eggs from inorganic and most organic materials in a sample. Terminal separation of eggs from minute, organic debris in water is currently done optically and is time consuming if many eggs are present.

A method developed at the Macon Mosquito Abatement District has greatly reduced the time.

METHODS AND MATERIALS. The new method of terminal separation follows the Illinois Egg Separation Procedure. In that procedure, eggs are separated from materials of dissimilar density by flotation in saturated solutions of NaCl in water and in water alone. The final result is a mixture of eggs and organic debris of similar density in the bottom of a clean casserole of water. The material within the casserole is viewed through a stereomicroscope and eggs are removed by pipette from surrounding debris.

The new method changes the optical separation procedure in three ways: (1) separation takes place in a casserole lined with paraffin wax, (2) the mixture of eggs and debris is heated prior to separation, and (3) a hydrogen peroxide solution in water is used instead of water alone.

Separation is made in a 150 ml porcelain casserole in which chips of paraffin wax have been melted and allowed to harden into a layer on the bottom. Bubbles may form on the bottom of a casserole during separation if not lined with wax. Wax should cover only the bottom of the casserole because wax on the sides will impede egg recovery. A wax lined casserole may be used for repeated separations.

The eggs and debris are heated by decanting water from the clean casserole containing the mixture and adding approximately 100 mls of 60°C to 70°C water. After several seconds the contents of the casserole are poured through a 100 mesh screen, trapping the eggs and debris. The heat treated mixture is washed with minimal water into the wax lined casserole.

The hydrogen peroxide solution is prepared by diluting commercially available 3% hydrogen peroxide solution with water. A 0.3% solution will usually give satisfactory separation, however, more concentrated solutions may be used. The solution is used while it is at room temperature (25°C). Solutions have been stored for 24 hr at room temperature without deterioration.

Approximately 100 ml of the diluted hydrogen peroxide solution is added to the wax-lined casserole containing the heat treated mixture. The action of hydrogen peroxide results in bubble formation within debris particles which are floated to the surface. Aedine eggs are not affected in this way. The contents of the casserole are gently spun to center the eggs on the wax and dislodge bubbles which may float some eggs. Eggs are pipetted from the

Table 1. Effect of exposure of field collected aedine eggs to hot water on rate of recovery in 0.3% solution of hydrogen peroxide.

Pre-separation treatment	Eggs Treated[a] No.	Percentage Eggs Recovered		
		Minimum	Maximum	Mean & S.E.
Not heated	1000	89	98	94.0 ± 2.9
Heated w/o drying	1000	94	100	98.6 ± 1.8
Heated and dried	1000	94	100	97.0 ± 1.6

[a] Number of replicates 10 for each treatment.

wax and placed in water to cleanse them of hydrogen peroxide prior to examination.

RESULTS AND DISCUSSION. Yields of eggs are uniformly high following separation from extraneous organic debris by the method described. Table I shows the effect of pre-separation treatments on the percentage of eggs recovered. Eggs not heated and assumed to be viable are not recovered as readily, or with equal regularity, as heat treated eggs. The percentage of recovery for heat treated eggs flattened by air drying is comparable to that for eggs heat treated but not dried.

Heating the eggs and debris prior to separation not only increases the likelihood of the eggs remaining submerged after addition of the solution, but also prevents eggs from hatching while under examination.

Several features of hydrogen peroxide separation make it adaptable to egg surveys for both abatement operations and investigative purposes. No appreciable difference has been observed in the percentage of recovery among eggs of aedine species commonly found in soil samples collected in Central Illinois (*Aedes vexans, Ae. trivittatus, Ae. sticticus, Ae. dupreei, Psorophora horrida, Ps. confinnis, Ps. ciliata*). Since nonviable eggs are also recovered using hydrogen peroxide separation, their relative frequency in a sample can be determined. Eggs intended for rearing may be separated using hydrogen peroxide if the recovery rate shown for eggs lacking pre-separation heat treatment is acceptable. Materials used in the procedure are relatively inexpensive and readily available. Above all, the method provides satisfactory terminal egg separation without the time consuming manipulation of the sample as required by optical separation.

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FURTHER OBSERVATIONS ON THE HOST RANGE OF THE MOSQUITO FUNGUS *CULICINOMYCES*

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Mosquito pathogenic fungi of the recently described genus *Culicinomyces* Couch, Romney and Rao have been discovered independently in Australia (Sweeney et al. 1973) and in the United States of America (Couch et al. 1974). A study of the Australian fungus has shown that it is pathogenic to larvae of Culicidae, Chironomidae, and Ceratopogonidae but not to some other aquatic insect larvae, the mosquito fish *Gambusia,* and freshwater shrimps (Sweeney 1975a). As these observations suggested that the susceptible hosts of this fungus may be restricted to certain dipterous families, an additional series of infection experiments was performed with aquatic larvae from other families of the Diptera.

Aquatic larvae of the families Tipulidae, Simuliidae, Stratiomyiidae, and Syrphidae were collected from fresh water habitats near Sydney and were transported to the laboratory