COLLECTION AND SEPARATION OF *AEDES TAENIORHYNCHUS* EGGSHELLS FROM MANGROVE SOIL[1]

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ABSTRACT. Two methods to separate eggshells of *Aedes taeniorhynchus* from mangrove soil were compared. Selective sieving, using nested sieves with 0.185 and 0.170-mm screen openings, and water flotation both removed over 99% of the soil. However, water flotation recovered a significantly greater percentage of eggshells (62% vs. 34%). There was no significant difference in the recovery rate of viable eggs and new and old eggshells using water flotation.

Mosquito eggshells provide a potential tool for elucidating the ovipositional history of aedine mosquitoes (Lopp 1957; S. A. Ritchie and D. S. Addison, unpublished data). Mosquito eggshells are relic chorions of hatched (hatch type) and unhatched (unhatched type) eggs found in the soil of oviposition sites; hatched eggshells consist of the relic egg chorion without the operculum. Upon finding large numbers of *Aedes taeniorhynchus* (Wied.) eggshells in mangrove soil (Ritchie and Johnson 1989), we initiated research to elucidate the relationship between eggshell populations and previous mosquito production. This paper reports 2 techniques for separating *Ae. taeniorhynchus* eggshells from mangrove soil.

Separation of aedine mosquito eggs (Horsfall 1956) and eggshells (Kay and Jorgensen 1986) from soil commonly involves sieving and flotation in a saturated salt solution (Horsfall 1956). Selective sieving isolates particles approximating the size of mosquito eggs, while suspension of sieved soil in a saturated salt solution then floats organic matter, including mosquito eggs, away from inorganic matter. Unfortunately, salt flotation proved ineffective in mangrove soils with a high organic content (Ritchie and Johnson 1989), necessitating the need for a new flotation technique.

Eggshells were collected from soil cores taken with a modified plastic syringe. The tip of a Monoject® disposable syringe (6 and 60-ml sizes) was cut off and the front edge of the barrel beveled on a grinder to produce a sharp cutting edge. The rubber tip of the plunger was cut off so that the plunger could be inserted into the syringe barrel with minimal resistance. Soil cores were taken to a depth of 2.5 cm for a core volume of 3 and 15 ml for the 2 syringe sizes, respectively. Individual cores were stored in 60-ml snap-top plastic vials.

Selective sieving (Horsfall 1956) was the first method tried for the isolation of eggshells from soil. The system consisted of nested sieves with the top sieve openings just large enough to allow passage of eggshells and the smaller bottom sieve openings adjusted to separate eggshells from smaller particles. Sieves were either of brass screen (13 cm diam, with 0.15 and 0.30-mm openings), or Nytex® screen (10 cm diam, with 0.170 and 0.185-mm openings). Nytex sieves were made by sandwiching a piece of screen between 10-cm diam male and female polyvinylchloride fittings then sealing the junction with silicon caulk.

Appropriate screen sizes were determined by placing eggshells (hatched and unhatched) on a sieve, then rinsing it with a shower head faucet and determining the percentage of eggshells washed through the sieve. In 8 trials (295 eggshells tested), 86.2 ± 3.9% (SE) of the eggshells passed through a 0.180-mm screen; thus, a slightly larger screen (0.185 mm) was used for the top sieve. Similarly, a 0.170-mm screen retained all eggshells so this size was used for the bottom sieve. Soil cores were washed vigorously through the sieves using a shower-head faucet for ca. 1 min and the soil collected in the lower sieve was rinsed into a 60-ml vial for storage. Eggshells were counted by pipetting the water-soil mixture into the wells of a Falcon® 24-well tissue culture plate then examining the culture plate wells, vial and pipette with a dissecting microscope.

The second method evaluated for recovery of eggshells was water flotation. We often observed eggshells floating on the water surface after sieving and speculated that the sculptured chorion of dried eggshells traps a thin layer of air that causes the eggshell to float. If so, sieved soil could be dried and suspended in water to float eggshells selectively from soil particles. By eliminating the majority of soil particles, sieves with larger openings could be used, thereby increasing the percentage of eggshells recovered, with-

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out increasing the amount of soil to be examined microscopically.

The following procedure was used to exploit water flotation as a means of separating *Ae. taeniorhynchus* eggshells from mangrove soil. Soil cores (15 mm) were rinsed through nested 0.30 and 0.15-mm sieves and the retained soil rinsed onto a 10 × 10 cm piece of 0.15-mm stainless steel screen. The screen was then placed on paper towel to remove moisture, then dried completely in a drying oven at 50°C for 24 h. The dried soil was removed and gently broken up with a ceramic mortar and pestle. The soil particles were rinsed onto a 0.15-mm sieve, wetted under a faucet then rinsed into a 1-liter separatory funnel containing ca. 100 ml of water. After 1 min, the separatory stopcock was opened until the settled soil was flushed out. The stopcock was then closed to retain any floating material and a wash bottle used to rinse down any soil stuck to the wall of the funnel. Again, any settled soil was similarly discarded. The floating soil remaining in the separatory funnel was rinsed onto 0.15-mm screen to remove water, then rinsed into a 60-ml vial for storage. Microscopy of eggshells was conducted as described earlier.

Two parameters were used to quantify the separatory effectiveness of the 2 methods. Sieving efficiency was defined as the percentage of soil removed from the original sample by the method. The more soil removed, the less that had to be examined microscopically and the more efficient the process. Eggshell recovery is the percentage of eggshells recovered by the process. Results are summarized in Table 1. All percentage data were arcsine transformed (Zar 1974) and analyzed for treatment differences with an unpaired *t*-test.

The sieving efficiency was compared for both methods. Soil cores (24, each of 15 ml) were collected from a mixed red mangrove (*Rhizophora mangle* Linn.) and black mangrove (*Avicennia germinans* Linn.) forest. Each core was placed in a 60-ml plastic vial, oven dried (50°C for 24 h) and then weighed. Water (5 ml) was then added to each vial to rehydrate the core.

Table 1. Percentage ($\bar{x} \pm$ SE) of soil and eggshells retained by selective sieving and water flotation (see text for methodology).

	Selective sieving	Water flotation
Soil retained (%) (15-ml core)	0.43 ± 0.07	0.60 ± 0.08
Eggshells retained (%) (3-ml core)	33.8 ± 4.4	62.0 ± 3.4
(15-ml core)	ND*	47.0 ± 2.1

* ND—not done.

Half of the cores ($n = 12$) were processed for eggshells using selective sieving and the remaining cores were processed using water flotation. The results (Table 1) indicated that although the sieving efficiency [(oven dry weight of sieved soil/oven dry weight of original sample) × 100] of the methods was significantly different ($t = 2.321$, $P = 0.032$), the difference was relatively minor (0.17%). The water flotation method, owing to a larger sieve opening size (0.300 vs. 0.185 mm), retained a significantly greater ($\bar{x} \pm$ SE) percentage of soil (7.16 ± 0.95%) from sieving than did selective sieving (0.43 ± 0.07%; $t = 6.76$, $P < 0.001$). However, water flotation removed a mean (± SE) of 90.7 ± 1.08% of this soil.

The percentage of eggshells recovered was estimated for both separation techniques. Eggshells (10 or 20 of both the hatched and unhatched type) were added to individual soil cores taken from a *R. mangle* forest presumed, on the basis of 45 3-ml samples that contained no eggshells, to be free of aedine mosquito eggshells. For selective sieving, 3-ml ($n = 32$) cores were used; for water flotation, 3-ml ($n = 15$) and 15-ml ($n = 10$) cores were used. The percentage of eggshells that passed through the sieve and was subsequently collected by water flotation also was determined. A significantly greater percentage of eggshells was recovered from the 3-ml ($t = 3.955$, $P < 0.001$) and 15-ml ($t = 2.909$, $P = 0.006$) cores using water flotation as opposed to selective sieving (Table 1). Of the eggshells that passed through the sieve, 86.1 ± 2.9% were recovered by water flotation. Because degradation of the eggshell chorion might affect its ability to float, the efficiency of water flotation to separate eggs and eggshells of different ages was tested. Groups of 10 viable eggs (from field collected females), new hatched eggshells (cylindrical black eggshells collected from eggs hatched that day) or old hatched eggshells (flat brown eggshells collected from mangrove soil) were placed separately within a 3-ml core of eggshell-free soil using a pasteur pipette then the soil core was processed by water flotation. The experiment was replicated 10 times and the number of eggs/eggshells recovered compared by a one-way analysis of variance. The respective mean (±SE) number of viable eggs, new eggshells and old eggshells recovered was 4.1 ± 0.8, 6.0 ± 0.4 and 5.3 ± 0.6 ($F = 2.420$, $P = 0.108$). A majority (31/41; 75.6%) of the viable eggs recovered was intact but collapsed. Only 2 noncollapsed eggs were recovered, although 6 viable eggs were found in the soil that sank. The results indicate that eggs and eggshells of different stages of degradation can be recovered at similar rates using water flotation.

Our experiments indicate that water flotation

provides significantly better recovery of *Ae. taeniorhynchus* eggshells of all age classes from mangrove soil than selective sieving. Water flotation collects a significantly greater percentage of eggshells without increasing substantially the soil retained. The method could be used effectively to process large soil samples that would be necessary to access the mosquito oviposition history of several sites. We have successfully used the method to isolate eggshells of other aedine mosquitoes [*Aedes infirmatus* Dyar and Knab and *Aedes vexans* (Meigen)] and feel it could be used successfully for others. Although water flotation can be used to isolate viable eggs and new eggshells, we believe that the sieving and bleaching method of Ritchie and Johnson (1989) and the hatching method of Bidlingmayer and Schoof (1956) require less labor and are more efficient.

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