USE OF SODIUM HYPOCHLORITE TO DETECT AEDINE MOSQUITO EGGS IN MANGROVE SOILS AND INSECT FECES[1]

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Sodium hypochlorite is a commonly used clearing agent in embryological studies of mosquitoes. The heavily pigmented chorion of aedine mosquito eggs necessitates clearing to allow observation of the embryo (Trpis 1970). Morterson (1950) first used commercial bleach (5% sodium hypochlorite) to facilitate observation of *Aedes nigromaculis* (Ludlow) embryos. Hokohama and Judson (1963), Beckel (1953) and Craig (1955) also used formulations of sodium hypochlorite to clear aedine mosquito eggs for study. The chorion, however, is somewhat resistant to bleaching; Morterson (1950) found that *Ae. nigromaculis* eggs withstood a 5% sodium hypochlorite solution for 6 min before clearing.

This scientific note reports the use of sodium hypochlorite to detect *Aedes taeniorhynchus* (Wied.) eggs in Florida mangrove forests.

Mangrove soil is highly organic and frequently contains rich peat deposits that harbor large populations of *Ae. taeniorhynchus* eggs (Ritchie 1984[4]). The egg separation method of Horsfall (1956) relies on selective sieving and salt flotation to remove eggs from soil. Unfortunately, the relatively low-density peat particles are not adequately separated from eggs by salt flotation. Red mangrove (*Rhizophora mangle* Linn.) peat was filtered through successive 0.25 and 0.15 mm (mesh opening size) filters to isolate eggs (Fig. 1A). The filtered material was then incubated in 2.5% sodium hypochlorite (50% commercial bleach) for 5 min at room temperature to see if peat could be selectively bleached to improve visibility of the eggs. The peat particles were selectively bleached such that mosquito eggs could be located without magnification (Fig. 1B). However, eggs can be "lost" if overbleached. The recommended procedure is to place the filtered soil in 50% bleach, for 3–5 min, stirring occasionally, until the soil turns brownish yellow. The solution is then poured into a 0.15 mm sieve and washed with water for 30 sec. This step removes much of the peat that has been partially dissolved by the bleach. Eggs should be counted immediately to avoid loss by overbleaching.

Mangrove peat often contained high densities of relic mosquito eggshells (inviable eggs and remnants of hatched eggs) that can be confused with viable eggs or eggshells from recently hatched eggs. Relic eggshells, typically faded in color, might clear faster than eggs that have been laid or hatched recently. Prolonged exposure to sodium hypochlorite might be used to selectively differentiate relic eggshells from viable eggs and fresh eggshells.

This was tested by comparing the clearing time of embryonated eggs and fresh and relic eggshells in 2.5% sodium hypoclorite (50% commercial bleach). Eggs collected from ovipositing wild *Ae. taeniorhynchus* females were incubated at room temperature for 1 week before testing. New eggshells were collected after hatching some of these eggs. Relic eggshells were collected from mangrove peat that had been passed through a 0.25 mm screen. A group of 10 eggs or 10 eggshells was placed in a watch glass and ca. 1 ml of 2.5% sodium hypochlorite added. The number of eggs or eggshells with any remaining chorionic pigment was tallied after a 5-min exposure. The experiment was replicated 10 times. On 4 occasions, the time for all 10 eggs to clear was measured.

Sodium hypochlorite appears to be an excellent medium for isolating fresh eggs and eggshells from relic eggshells. Relic eggshells cleared significantly faster [$P < 0.01$, *t*-test (Schlotzhauer and Littell 1987)] than fresh eggs and fresh eggshells. The percentage of relic eggshells clearing within 1, 2.5 and 5 min was 12, 50 and 100%, respectively. Only 2 of 100 fresh eggs cleared in 5 min; both of these eggs were infertile. The time ($X \pm SD$) for 4 groups of 10 eggs to clear completely was 47.8 ± 6.9 min. No significant differences were found for the fresh eggs vs. fresh eggshells comparison. This experiment indicates that a 5-min exposure to 50% commercial bleach is sufficient to remove most relic eggshells from viable eggs.

A study was conducted to see if 5% sodium hypochlorite could verify if an animal had ingested *Ae. taeniorhynchus* eggs. Several hundred

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[4] Ritchie, S. A. 1988. A simulation model of the population dynamics of the black salt marsh mosquito (*Aedes taeniorhynchus*) in a Florida mangrove forest. Ph.D. Dissertation, Univ. of Florida. Gainesville, FL.

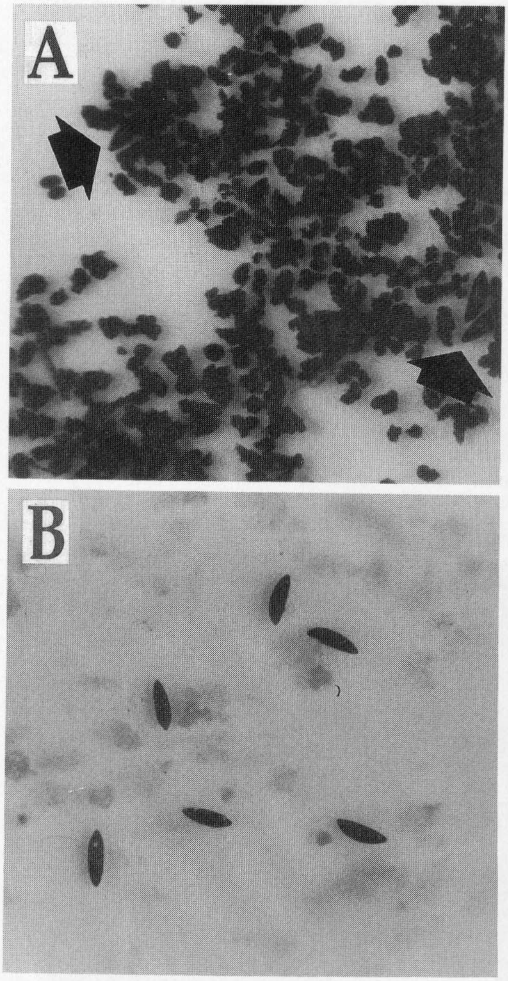

Fig. 1. A) Unbleached, sieved (through successive 0.25 and 0.15mm sieves) mangrove peat containing *Aedes taeniorhynchus* eggs (marked by arrows); B) Same peat following a 5-min incubation in 2.5% sodium hypochlorite (50% commercial bleach); eggs can be readily seen with the naked eye.

Ae. taeniorhynchus eggs and a small piece of Purina® cricket chow were placed in a 100-ml plastic container containing three adult *Gryllus* sp. After 48 h, cricket feces were collected and a drop of 5% sodium hypochlorite was added. Bleaching was facilitated by teasing the feces apart with forceps. Although egg fragments can be seen in unbleached feces by their dark metallic sheen and sculptured surface (Craig 1955), bleach facilitates the ability to quickly spot suspected egg fragments (Fig. 2) by dissolving and lightening fecal material. Screening must be done quickly because the small egg fragments bleach faster than whole eggs or eggshells. This method, although not applicable to piercing-sucking and extraoral feeders, should be more practical than radioisotope (James 1966) and serological (Service 1973) techniques used to verify that an insect has ingested mosquito egg(s).

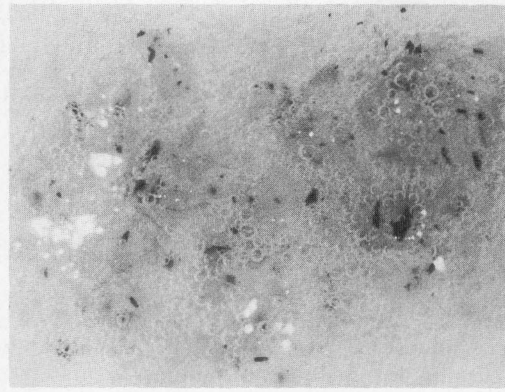

Fig. 2. *Gryllus* sp. feces after a 2-min exposure to 5% sodium hypochlorite. Small, dark particles are chorion fragments from *Aedes taeniorhynchus* eggs that were ingested by the cricket ca. 48 h earlier.

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