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Examination of a Miniaturized Funnel Trap for *Aedes aegypti* (Diptera: Culicidae) Larval Sampling

R. L. Burke¹, R. Barrera², T. Kluchinsky^{3,4}, M. Lewis⁴, and D. M. Claborn⁵

R. L. Burke: ronald.l.burke@us.army.mil

¹Armed Forces Health Surveillance Center, Division of Global Emerging Infections Surveillance and Response System, 503 Robert Grant Ave., Silver Spring, MD 20910

²Centers for Disease Control and Prevention–Dengue Branch, 1324 Calle Cañada, San Juan, Puerto Rico 00920

³U.S. Army Public Health Command (Provisional), 5158 Blackhawk Rd., Aberdeen Proving Ground, MD 21010

⁴Uniformed Services University of the Health Sciences, 4301 Jones Bridge Rd., Bethesda, MD 20814

⁵MPH Program, Missouri State University, 901 S. National Ave., Springfield, MO 65897

Abstract

Funnel traps are often used to sample for the presence of *Aedes aegypti* (L.) (Diptera: Culicidae) larvae in subterranean aquatic habitats. These traps are generally 15 cm in diameter, making them impractical for use in subterranean sites that have narrow (10-cm) access ports, such as those in standard-sized septic tanks. Recent research indicates septic tanks may be important habitats for *Ae. aegypti* in Puerto Rico and the Caribbean. To sample mosquito larval populations in these sites, a miniaturized funnel trap was necessary. This project describes the use of a smaller funnel trap for sampling larval populations. The effects of larval instar (third and fourth) and population density on trap efficacy also are examined. The trap detected larval presence 83% of the time at a larval density of 0.011 larvae per cm² and 100% of the time at densities 0.022 larvae per cm². There was a significant trend of increasing percentage of recaptured larvae with higher larval population densities. Although the miniaturized funnel trap is less sensitive at detecting larval presence in low population densities, it may be useful for sampling aquatic environments with restricted access or shallow water, particularly in domestic septic tanks.

Keywords

Aedes aegypti; septic tanks; funnel trap

Correspondence to: R. L. Burke, ronald.l.burke@us.army.mil.

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Subterranean wells (Russell et al. 2002), sewers (Gonzalez and Suarez 1995), and septic tanks (Babu et al. 1983, Barrera et al. 2008) are recognized as potential sites for *Aedes aegypti* (L.) (Diptera: Culicidae) productivity and larval development. Traditional larval survey methods such as visual inspection, sweep nets, and dipping are often impractical due to the limited access associated with these habitats. Instead, investigators have used floating funnel traps as a passive means of sampling immature mosquito populations in underground aquatic habitats (Harrison et al. 1982, Kay et al. 1992, Gionar et al. 1999, Russell and Kay 1999, Nam et al. 2003). Advantages of the funnel trap include its simple construction (inverted funnel, well, and sinker) and low material cost. However, these studies have shown that larval instar, larval population density, and trap depth can affect the effectiveness of the funnel trap in catching larvae.

A 2006 study by the Centers for Disease Control and Prevention (CDC) found many *Ae. aegypti* adults emerging from septic tanks in the Puerto Rican community of Playa-Playita (Barrera et al. 2008). A follow-up study was conducted between February and April 2008 to document the presence of larvae within the septic tanks and to look for environmental factors associated with their presence (Burke et al. 2010). Unfortunately, access ports in many of the septic tanks were <15 cm in diameter; thus, they were too small to allow the use of previously validated funnel traps. As a result, researchers used a miniaturized version of the previously calibrated Vietrap (Russell et al. 2002). This study reports on the validation of the miniaturized trap as a sampling device for subterranean mosquito larval populations and on the effects of larval instar and population density on the performance of the modified trap. The trap was partially described in a previous publication (Burke et al. 2010); however, this short communication provides a detailed description of trap construction and the results of laboratory testing of trap efficacy.

Materials and Methods

Funnel Traps

The miniaturized funnel trap was constructed by drilling a hole in the lid of a 120-ml plastic urine specimen cup (1700 Series 4 oz Graduated Container, Kendall Healthcare, Mansfield, MA) into which the tip of a polypropylene funnel was then inserted to project into the specimen cup (Fig. 1). The specimen cup was 7.3 cm in height, with lid diameter of 6.7 cm and a tapered base diameter of 5 cm. Each funnel was 11.5 cm in height, including a 4.5-cm stem, with a 10-cm mouth and 1-cm stem opening. The funnel was held in place with two size 4, 0.95-cm (3/8-in.) screws that were partially inserted into the funnel neck. A 3.65-cm o.d. (2.9-cm i.d.) by 0.48-cm steel locking washer (26 g) was placed around the neck of the funnel as a counterweight, before the funnel's insertion through the lid. When filled, the trap had an 11.5-cm draft and an overall height of 14.5 cm. The original Vietrap had an opening diameter of 18.5 cm and overall draft of 18 cm. The original design also had two self-tapping screws in the funnel neck and a 2-cm section of galvanized pipe (4.5 cm o.d.) was used as counterweight instead of the locking washer.

For trapping operations, the specimen cup was filled to four-fifths capacity and lowered into the experimental pool water described below. The trap inverted upon entering the water

which allowed larvae to swim up through the funnel and subsequently become trapped within the cup.

Pool Tests

Three trapping replicates were run for two larval instars, third and fourth instars, at four larval densities: 200 larvae (0.011 larvae per cm²), 400 larvae (0.022 larvae per cm²), 600 larvae (0.033 larvae per cm²), and 1,000 larvae (0.055 larvae per cm²). Two 1.5-m-diameter, plastic wading pools were used for larval trapping. The pools were filled with tap water and left at room temperature for 1 h before the initial introduction of larvae on the first day. The depth of the water was 24 cm, and the water temperature was checked each morning ($20.4 \pm 0.3^{\circ}\text{C}$). The pools were placed adjacent to each other, directly under fluorescent ceiling lights, which were on from $\approx 7:00$ a.m. to 6:00 p.m. Larval instars were rotated between pools each day (e.g., third instars pool A on day 1, pool B on day 2).

Mosquito Larvae

Larvae were hatched from *Ae. aegypti* Rockefeller strain eggs that were obtained from the CDC insectary in San Juan, Puerto Rico. Larvae were reared in 475-ml plastic cups (50 larvae per cup), then they were sorted by instar and recounted before transferring them to the pool. Equal numbers of larvae were added to each quadrant of the pool in an attempt to minimize potential clustering of the larvae in one quadrant. Larvae were left in the pools with the floating funnel trap for 24 h, after which time both the trap and all remaining larvae were removed from the pool.

Data Analysis

StataIC 10 (StataCorp LP 2007) was used for statistical analyses. The Wilcoxon signed rank test was used to compare the trapping percentages between larval instars. Daily trap counts were converted to the percentage of larvae trapped out of the total larval population for each instar. The difference in trap catch percentage (percentage of third instars trapped minus the percentage of fourth instars trapped) was calculated for each population density and a Kruskal–Wallis equality of populations rank test was used to examine the differences between trap percentages to assess the interaction between larval instar and population density. A nonparametric trend test (an extension of the Wilcoxon rank sum test) was used to assess the effect of population density on the percentage of trapped larvae.

Results

The miniaturized trap detected larval presence (defined as at least one recaptured larvae present in the trap upon retrieval after 24 h) 83% of the time with a larval population density of at least 0.011 larvae per cm² (Table 1). At larval population densities ≥ 0.022 larvae per cm² the trap detected larval presence 100% of the time. There was no significant difference in the percentage of trapped larvae between third and fourth instars (Wilcoxon signed rank, $Z = 0.784$, $P > 0.43$). The interaction between larval population density and larval instar was also not significant (Kruskal–Wallis, $\chi^2 = 0.897$, $df = 3$, $P = 0.83$). Because there was no significant difference between the trapped percentages between third and fourth instars, these results were subsequently combined before examining the trend in trapped percentage

of larvae across larval population densities and calculation of expected trap catches based on the larval population. There was a significant (nonparametric trend test, $Z = 3.04$, $P = 0.002$) trend of increasing trap effectiveness (higher percentage trapped) with higher larval population densities for both larval instars.

Discussion

The increased percentage of recaptured larvae at higher larval populations was unexpected, as two previous funnel trap reports for *Ae. aegypti* and *Culex quinquefasciatus* (Say) did not note an increasing trend in the percentage of trapped larvae with increasing population densities (Kay et al. 1992, Russell and Kay 1999). The increased performance noted in this study may be due to the clustering behavior of the larvae. Under laboratory conditions, *Ae. aegypti* larvae have been noted to congregate in small sections of rearing pans (McLean-Cooper et al. 2008). With high densities, there is an increased probability of interlarvae encounters that may increase larval movement, thereby increasing the likelihood of larvae entering the funnel trap. Other potential reasons for the observed differences between this study and previously published results include differences in methodology such as trapping duration, water depth, water temperature, and lighting. However, further study is needed to examine this hypothesis and the reason behind the observed differences between this report and the previous reports.

The results of this study suggest the miniaturized funnel trap may be less sensitive for detecting *Ae. aegypti* larval presence than its larger counterparts. Although the miniaturized trap examined in this study was 83% sensitive at detecting larval presence at a density of 0.011 larvae per cm², at least two other larger funnel traps were 100% sensitive for *Ae. aegypti* larval presence at densities as low as 0.002 larvae per cm² in similarly sized (1.2-m-diameter) containers (Russell and Kay 1999). Despite the implications associated with a decreased sensitivity for detecting larval presence at low densities, it is important to recognize the potential benefits of using a smaller trap. The smaller funnel opening diameter and shorter draft allow usage in aquatic environments with restricted openings or in shallow waters. Also, a shorter draft was noted previously to improve trap performance (Russell and Kay 1999). Although the miniaturized funnel trap is less sensitive than larger versions, it would still be useful for larval surveillance, especially in septic tanks where small access ports prevent the use of other sampling methods. The demonstrated presence of *Ae. aegypti* larvae in the septic tanks of Puerto Rico renders further investigation of this habitat and its importance in the possible maintenance or transmission of dengue necessary (Burke et al. 2010). The miniaturized trap described here provides a broader capability for monitoring subterranean mosquito populations. This trap also was constructed using standard components readily available from medical distributors that should facilitate easy replication in subsequent studies.

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Fig. 1. Miniaturized floating funnel trap for use when sampling for mosquito larvae in subterranean aquatic habitats with small openings. A hole is drilled through the lid of a 120-ml specimen cup (A) and the tip of an 11.5-cm plastic funnel (B) is inserted. The specimen cup has a height of 7.3 cm, a lid diameter of 6.7 cm, and a tapered base with a 5-cm diameter. The lid is held in place by the partial insertion of two screws (C) into the funnel neck. A 3.65-cm o.d. metal washer (D) is placed around the funnel neck as a counterweight before attaching the cup lid and screws. When constructed the trap has an opening diameter of 10 cm, a

minimum draft of 11.5 cm, and an overall height of 14.5 cm. Figure is drawn to scale as shown.

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Table 1

Number (mean $\pm$ SD) of *Ae. aegypti* larvae recaptured and percentage of total larval population recaptured using a miniaturized funnel trap from a 1.5-m-diameter pool at varying larval densities

Larval density ^a	Larvae recovered ^b	% recaptured	Trap detection rate of larval presence (%)
(0.01)	7.2 $\pm$ 10.9	4 $\pm$ 5	83
(0.02)	39.7 $\pm$ 41.9	10 $\pm$ 10	100
(0.03)	77.83 $\pm$ 38.1	13 $\pm$ 6	100
(0.06)	211.2 $\pm$ 119.2	21 $\pm$ 12	100

^aLarvae per cm².

^bMean of 6 replicates.