

## AN EFFICIENT FLOATING LARVAL TRAP FOR SAMPLING *Aedes aegypti* POPULATIONS (DIPTERA: CULICIDAE)<sup>1</sup>

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**Abstract.** A floating mosquito-larval trap for medium- to large-sized artificial containers was developed that eliminates many of the biases commonly associated with human collection techniques. This trap was extremely efficient in sampling *Aedes aegypti* and *Culex quinquefasciatus* immatures during both laboratory and field tests. Field trials revealed that the AFRIMS trap was highly sensitive (98.4 to 99.2%) for detecting immatures in known positive water jars in a 24-h period. In addition, over 42,000 immatures were collected in 1322 traps, and results from 1159 traps in which larvae and pupae were separated revealed an average of 31 larvae and 1.4 pupae collected per trap per 24-h period. Other advantages of the trap besides efficiency and sensitivity are its small size, low cost and simple construction. It keeps specimens alive, collects larvae and pupae, is sturdy and durable, functions as a random sampler, and also can be used as a surveillance device.

In many Southeast Asian countries, water for household use is stored primarily in clay-ceramic jars, cement tanks or basins, 250-l metal drums and other artificial containers. These containers commonly serve as important habitats for immature *Aedes aegypti* (L.), the primary urban vector of dengue viruses. In Thailand, water jars inside and adjacent to households may be the single most important habitat for this species (Tonn et al. 1969). Accordingly, surveys and estimates of the population density of immature *Ae. aegypti* in this and other types of artificial containers are required for most research and control programs for dengue in Southeast Asia.

Standard survey methods for immature *Ae. aegypti* range from the "one-larva-per-container" method (Sheppard et al. 1969) through methods

using a given number of dips per container, to a total count of immatures (Chan et al. 1971) used for small containers. Such methods have been used in determining the various "classical" *Ae. aegypti* larval indices reviewed by Service (1976), i.e., house index, container index and Breteau index, or the larval density index (Chan et al. 1971). Except for the last, these allow only very crude estimates of *Ae. aegypti* density.

Since December 1977 we have been involved in a major epidemiological study of urban dengue in Bangkok. One goal of this study was to develop a sampling device for immature *Ae. aegypti* that would eliminate human bias associated with the classical dipping collections, and that also might provide a reliable technique for determining relative densities. The development and the laboratory and field testing of a floating larval trap are described below.

### MATERIALS AND METHODS

This floating, inverted-funnel trap is similar to an earlier trap (Muul et al. 1975) used for collecting mosquito larvae in ground (stump) holes in the United States, which was modified and developed into the present trap at the Department of Medical Entomology, Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok, Thailand. The mechanism for the trap was based on the premise that with repeated dives, mosquito larvae and pupae would eventually return to the surface through the trap funnel. The current (AFRIMS) trap (Fig. 1) is ca. 15 cm tall and 15 cm wide and will float in as little as 14 cm of water. The components of the trap (Fig. 2) consist of: (1) a round, clear plastic kitchenware container 13 cm diam × 6 cm deep, with a removable lid (1 hole is required in the center of the bottom for the insertion of the funnel neck and another at the edge of the lid for the insertion of a rubber stopper; both the funnel and the stopper should fit snugly in the holes); (2) a white or light-colored funnel ca. 12 cm long, with the mouth end 10 cm wide, the funnel acute angle about 50° and a hole ca. 1 cm diam in the funnel neck (the funnel neck is inserted up through the

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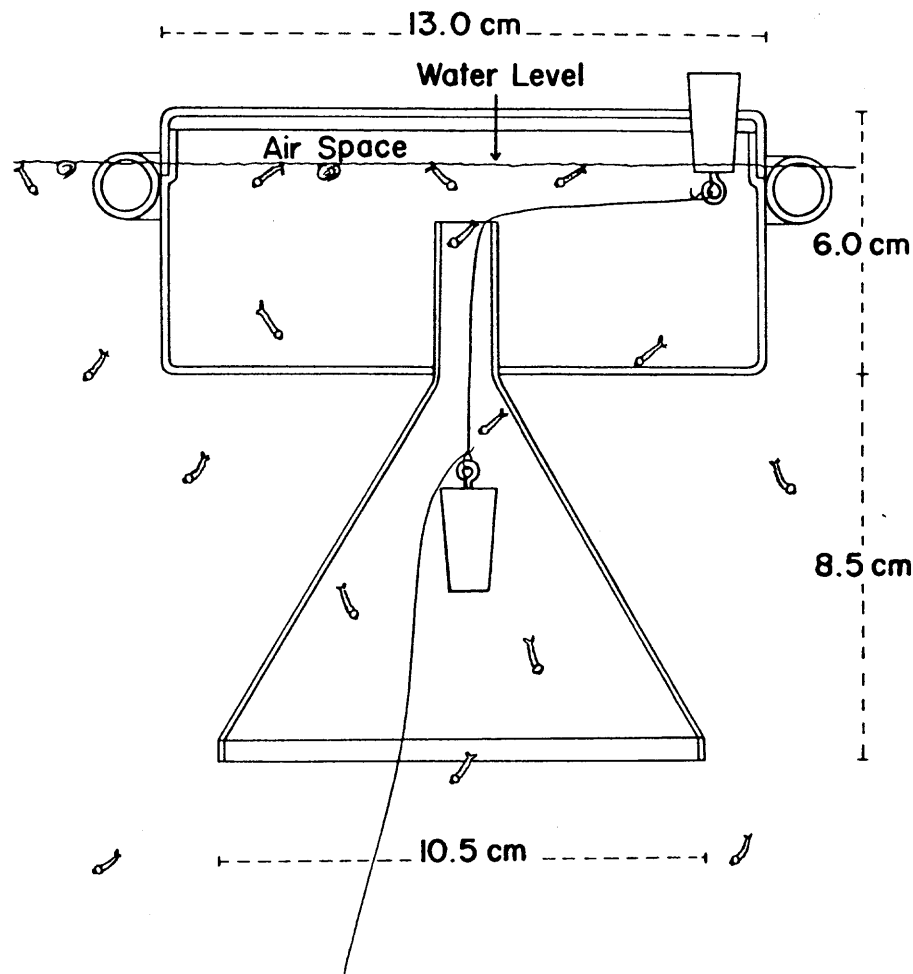


FIG. 1. Dimensions and lateral view of AFRIMS trap when set.

bottom of the container); (3) 2 rubber stoppers that fit snugly into the holes in the container lid and the funnel neck; (4) plastic hosing ca. 1.3 cm in diam and 44 cm long, which will join together to fit snugly around the container to serve as a float collar; (5) 2 metal screw eyes to insert into the 2 rubber stoppers; and (6) strong monofilament fishing line to run through the assembled trap and attach to the screw eyes in the 2 rubber stoppers.

To set the trap: (a) the container lid is secured to the container bottom by removable tape and the rubber stopper is pulled out of the hole in the lid; (b) the bottom stopper in the funnel is pulled down to assure that the funnel neck is open and that water can flow in; (c) the trap is lowered, funnel down, into the water and filled through the funnel neck until the water level is approximately even with the top of the collar float and above the top of the funnel neck inside the trap (Fig. 1); and (d)

the top stopper is then pushed snugly down into the hole in the lid, trapping air in the top of the container. The air inside the trap and the plastic tube collar cause the trap to float.

To remove the trap from the water: (a) the trap is held in a floating position in the water with one hand and the rubber stopper in the lid is pulled out and up, pulling the lower stopper snugly into the funnel neck and trapping the water inside; and (b) trapped larvae and pupae can then be emptied into other containers by pouring the water out through the hole at the edge of the container lid. At this point the trap can be stored or reset.

All laboratory tests were conducted for 24-h periods in 32-l and 54-l clay-ceramic jars in a screened room. The water levels in the 32-l and 54-l jars were adjusted to the 20-l and 42-l level, respectively, for the tests. This permitted the trap, which covers ca. 132 cm<sup>2</sup> of water surface area, to float

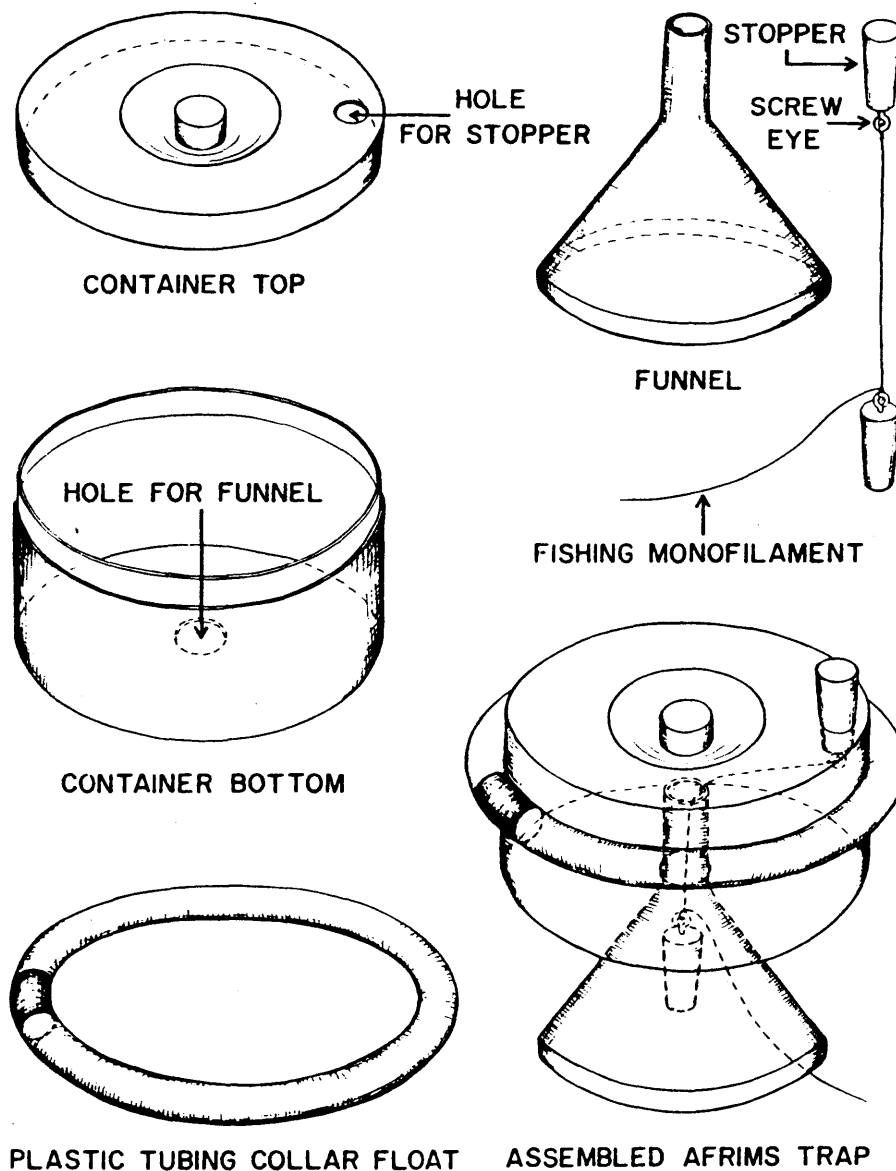


FIG. 2. Components of AFRIMS trap and view when assembled.

randomly within the 2 different-sized surface areas, i.e., 1104 cm<sup>2</sup> and 1661 cm<sup>2</sup>. The water depths of the 32-l and 54-l jars at the adjusted test levels (above) were 22 cm and 26 cm, respectively.

Evaluation of the trap sensitivity and efficiency for each size jar was based on 5 replicates, with 10 jars run simultaneously per replicate, i.e., 5 jars each with 100 3rd- and 4th-instar *Aedes aegypti* (Bangkok strain) and 5 jars each with 100 3rd- and 4th-instar *Cx. quinquefasciatus* (Bangkok strain). The trap was tested in 32-l jars on 4, 8, 11, 16 and 18 August 1978, and in 54-l jars on 12, 13, 17, 18 and 20 October 1978. Ambient temperatures during

the 1st period ranged from 25.2–33.2 °C, while those of the 2nd period were 23.0–33.0 °C. Approximately 48 h prior to a test the jars were filled with tap water. Larvae and 1 trap were placed in each jar at 1400 h on test days. The trap was removed and the captured larvae/pupae were counted at the same time on the following day. When the traps were introduced into the jars, care was taken not to capture larvae. The jars were not disturbed during the 24-h test period. Immatures remaining in the jars after removal of the traps were collected and counted to confirm the number of specimens captured.

TABLE 1. *Aedes aegypti* and *Culex quinquefasciatus* larvae captured by the AFRIMS trap in water jars during 24-h laboratory tests.

	JARS*		TOTAL	NO. LARVAE CAPTURED		
	SIZE	NUMBER**		RANGE PER JAR	RANGE IN REPLICATE MEANS (%)	TEST MEAN (%)
<i>Aedes aegypti</i>	32-l	25	1368	18-80	37.4-69.0	54.7
<i>Culex quinquefasciatus</i>	32-l	25	1502	24-88	55.8-69.2	60.1
<i>Aedes aegypti</i>	54-l	25	1152	20-82	37.0-63.0	46.1
<i>Culex quinquefasciatus</i>	54-l	25	1612	36-85	44.4-79.0	64.5

\* Each jar provided with 100 3rd-4th-instar larvae before each test.

\*\* Tests for each jar size consisted of 5 replicates, with 5 jars per species run simultaneously per replicate.

The trap was evaluated in natural mosquito immature habitats during a dengue ecology and epidemiology study in the Din Daeng area of Bangkok between Jan. 1979-Dec. 1980. Traps were placed in various sized water jars positive for immature mosquitoes for 24-h periods in up to 100 residences during each of 17 three-week survey periods. The number of traps set (1 per jar) varied per residence and families were encouraged not to disturb the traps, in hopes of reducing repeated diving reactions by the mosquito immatures that could bias results.

#### RESULTS

In replicated laboratory tests the AFRIMS traps were very efficient, consistently capturing between 37-69% (means) of the *Ae. aegypti* larvae, and between 44-79% (means) of the *Cx. quinquefasciatus* larvae in the 32-l and 54-l water jars (Table 1). The trap also demonstrated a 100% "sensitivity" in the laboratory tests. An independent *t*-test was used to determine if the water surface areas in the 2 different-sized jars had an effect on the trapping results. Significantly more ( $t < 0.015$ , 48 df) *Ae. aegypti* larvae were trapped in 32-l jars ( $\bar{x} = 54.7\%$ ) than in 54-l jars ( $\bar{x} = 46.1\%$ ). However, the number of *Cx. quinquefasciatus* larvae trapped in the 32-l jars ( $\bar{x} = 60.1\%$ ) was not significantly different from the number trapped in the 54-l jars ( $\bar{x} = 64.5\%$ ). Also,

a paired *t*-test was used to compare the trapping success for *Ae. aegypti* and *Cx. quinquefasciatus*. There was no statistical difference for these species in the 32-l jars, but in the 54-l jars significantly more ( $t < 0.001$ , 24 df) *Cx. quinquefasciatus* were caught.

During the 2 years of field trials, traps were placed in 1322 known positive water jars and 42,541 immature mosquitoes were captured, of which over 98% were *Ae. aegypti*. Of the 1322 traps set, only 21 failed to collect immature mosquitoes in the 24-h period, indicating a trap "sensitivity" (Dixon & Massey 1969) of 98.4%. During the 1st 2 field survey periods the 5305 larvae and pupae collected in 163 traps were not separated; thus, data on trapping effectiveness for these 2 life stages (Table 2) were based on 1159 traps set during the following 15 survey periods. The "sensitivity" of the trap during these 15 survey periods was 99.2%, with larvae constituting 95.7% of the immatures collected. These data (Table 2) indicate that a mean of 30.99 larvae and 1.39 pupae were collected per positive AFRIMS trap in 24 h. More specific *Ae. aegypti* density data from the AFRIMS trap in the Din Daeng study area will be published elsewhere.

#### DISCUSSION

Differences in the trapping results for *Ae. aegypti* in the 2 sizes of water jars, and between *Ae. aegypti* and *Cx. quinquefasciatus* in the 54-l jars, may be at-

TABLE 2. Results of field trials with AFRIMS traps set in mosquito-positive water jars in the Din Daeng area, Bangkok, during 15 survey periods in 1979-1980.

	TRAPS			MOSQUITOES COLLECTED*		
	TOTAL	POSITIVE	NEGATIVE	LARVAE	PUPAE	TOTAL
No.	1159	1150	9	35,637	1599	37,236
%	100	99.2	0.8	95.7	4.3	100

\* Over 98% *Ae. aegypti*.

tributed to behavioral differences in the larvae of these 2 species. *Aedes aegypti* larvae are plankton and scavenger feeders (Harbach 1977) and often feed on the sides or bottom of the jar. They tend to concentrate on the darkest side of the jar and seldom congregate around floating objects on the water surface. In contrast, *Cx. quinquefasciatus* larvae are mostly plankton feeders (Harbach 1977) that are less active, tend to congregate around objects floating on the surface, and are not usually influenced by minor light differences. Thus, the trap should be less effective for *Ae. aegypti* larvae in large containers because of their more random behavior, while the lack of significance in the number of *Cx. quinquefasciatus* collected in the 2 different-sized jars is probably due to the trap serving as an attractant for these larvae. These behavioral differences may also explain the results of the paired *t*-tests comparing the 2 species in the 2 different-sized jars. Actually, the trap appears to be more effective for *Cx. quinquefasciatus* than for *Ae. aegypti*.

Both field and laboratory data revealed that the AFRIMS trap was highly efficient and sensitive for detecting *Ae. aegypti* and *Cx. quinquefasciatus* immatures. Efficiency refers to the ability of the trap to collect adequate numbers of specimens for statistical analysis, while sensitivity is a measure of the ability of the trap to detect infested jars. These features suggest that this trap would be effective for mosquito surveillance, particularly in areas where large water containers represent a significant mosquito larval habitat. During 21 survey periods between 1978–1980 in the field study area, from 66 to 94% ( $\bar{x} = 82\%$ ) of the *Ae. aegypti*-positive houses identified during a given container survey period could be identified as positive by inspections of water jars alone. In this situation, the AFRIMS trap could easily and accurately function as a routine surveillance device. Besides the above, the trap has the following additional advantages. (1) The 42,541 immature mosquitoes collected during the field trials in only 1301 traps represent an adequate sample for determining the relative population densities of *Ae. aegypti*. Catches of 100–300 specimens per trap for a 24-h period were common, and over 700 specimens were collected in 1 trap. (2) The trap functions as a random sampler, providing an equal chance for the capture of immatures. Bias attributed to human search and collection techniques is effectively eliminated, provided the person setting the trap is careful not to

suck immatures into the trap while it is being set. (3) The trap size described here will permit its use in a variety of medium- to large-sized artificial containers or even in large, deep ground pools, sewage lagoons or small ponds. As presently designed, the trap will float in 13–14 cm of water; however, the depth needs to be slightly deeper to allow the larvae to get under the funnel. (4) The trap is simple, easily assembled and costs approximately US\$0.90 per copy in Thailand. (5) Pupae and all 4 larval instars are collected in the trap. Fewer 1st- and 2nd-instar larvae are collected because they often remain near the water surface and do not dive as deeply when disturbed. (6) Specimens captured in the trap remained alive for at least 48 h. During the present study, survival did not present a problem as all traps were in jars (inside or outside houses) and were exposed to sunlight only briefly or not at all. However, during an earlier study (Muul et al. 1975), larvae in traps in ground (stump) holes exposed to sunlight were frequently killed if left in the trap during the day. (7) During 2 years of field trials the trap proved to be very sturdy and durable. The traps also proved to be very stable, due primarily to the combination of the funnel weight, the position of the collar float and the closed top. (8) Psychologically, the trap is beneficial because the owner can see the results (removal of the mosquito immatures) and this usually outweighs the disadvantage of having to put the trap into the jars. (9) Although the AFRIMS trap was originally designed as an unbiased density sampling device, its efficiency is such that it may function very well as a control device.

Several disadvantages were noted for the trap. (1) The AFRIMS trap requires a number of hours and 2 trips to the container to sample the immature population in a container. (2) The trap is too large for use in such well-known and important *Ae. aegypti* habitats as ant traps, vases, tires, small plastic containers and most footbaths. The size could be reduced, but the trap still would not function in all of these habitats. (3) While the trap eliminates most possibilities for human sampling bias, it probably serves as an attractant to *Cx. quinquefasciatus* larvae and possibly also attracts some *Ae. aegypti* larvae. Although this is a bias for random samples, it would prove beneficial for a control device.

The AFRIMS trap is an extremely efficient surveillance tool and sampling device for *Ae. aegypti* and *Cx. quinquefasciatus* larvae and pupae in mod-

erate- and large-sized artificial containers, and it may be valuable as a control device in certain situations. Because of size limitations the trap cannot be used as the sole indicator for the classical "Breteau," "Container (Receptacle)," "Infested (Receptacle)" and "House (Premise)" indices. The major advantage of this trap is its elimination of nearly all of the biases commonly associated with human search and collection techniques. Accordingly, the AFRIMS trap should be of considerable value in determining mosquito species composition and relative densities in artificial containers during control or research programs.

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