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QUANTITATIVE EVALUATION OF FUNNEL TRAPS FOR SAMPLING IMMATURE *AEDES AEGYPTI* FROM WATER STORAGE JARS

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ABSTRACT. The trend is increasing to incorporate assessments of abundance into surveys for immature *Aedes aegypti* to identify the most important types of containers that should be targeted for control. In this study, we examined whether funnel traps could be used to sample immature *Ae. aegypti* from water storage jars ranging in size from 0.28-m diameter (30 liters) to 0.52-m diameter (150 liters). The effects of jar size and duration of funnel trap sampling were investigated and a set of calibration factors was developed to convert funnel trap numbers to absolute population estimates (0.28-m diameter = 2.5, 0.38-m diameter = 3.0, 0.48-m diameter = 4.6, and 0.52-m diameter = 7.4). Although the funnel traps were highly sensitive (90–100%) for detecting immature *Ae. aegypti* at densities as low as 25 3rd and 4th instars per jar, the large variation in funnel trap recapture rates meant that absolute population estimates based on a single funnel trap sample were inaccurate. However, by using a computer simulation, estimates of the total overall numbers of larvae from multiple jars were reasonably accurate (±20%), if more than 50 positive jars were surveyed. For example, 95% confidence intervals for the percentage error in estimated numbers of immatures from a series of 50 0.38-m-diameter and 50 0.52-m-diameter jars, were −10.0% to +10.2% and −19.9% to +17.8%, respectively. Although we generally recommend the use of nets to sample immature *Ae. aegypti* in jars, under some conditions funnel traps may be more acceptable than nets, because some householders object to the increased turbidity associated with net sampling in jars.

KEY WORDS *Aedes aegypti,* funnel traps, jars, quantitative sampling

INTRODUCTION

Traditional indices of abundance of *Aedes aegypti* (L.) such as the Breteau index (Breteau 1954), house index (Connor and Monroe 1923, Dunn 1923), and container index reflect the prevalence of containers infested with *Ae. aegypti.* Therefore, not surprisingly, only weak relationships exist between these indices and absolute densities of *Ae. aegypti* and risk of dengue transmission (Sulaiman et al. 1996, Focks and Chadee 1997). Because of this, the trend is increasing to incorporate assessments of immature abundance into survey methodologies (Tun-Lin et al. 1995), by using direct counts of immatures in small containers (flower vases, ant traps, bottles, and tins), or through the use of nets to sample immatures from large containers such as jars, drums, and tanks (Tun-Lin et al. 1994, Romero-Vivas et al. 2002) and funnel traps to sample underground containers such as wells and flooded, disused mine shafts (Kay et al. 1992, Jennings et al. 1995, Russell et al. 1996). In some cases, calibration factors have been applied to net and funnel trap catches (Russell and Kay 1999) to estimate the number of immatures in these large containers.

Although the funnel trap was originally designed for sampling immature *Ae. aegypti* from wells and other underground containers, in this study we examined whether funnel traps may have broader use in sampling *Ae. aegypti* from water storage jars ranging in size from 0.28-m diameter (30 liters) to 0.52-m diameter (150 liters). Because field staff often are required to survey large numbers of water jars and then identify the individuals collected, we based our assessments on 3rd and 4th instars because they were easier to identify than 1st and 2nd instars. Mortality is also high in 1st and 2nd instars and therefore numbers of immatures may not reflect the numbers of adult mosquitoes that emerge (Southwood et al. 1972). Although numbers of pupae are more closely associated with adult abundance, compared with 3rd and 4th instars, and therefore risk of dengue transmission (Focks and Chadee 1997), pupae are difficult to identify and this necessitates the rearing of adults for identification. Therefore, based on laboratory trials involving 3rd and 4th instars, we calculated a series of calibration factors to convert funnel trap catches to absolute population estimates. A computer simulation model was used to evaluate whether funnel trap sampling was an accurate method of estimating the total number of immatures in a series of jars, such as is required for community-based surveys of abundance of *Ae. aegypti.*

MATERIALS AND METHODS

Funnel traps: Each funnel trap consisted of a 185-mm-diameter plastic funnel, a 500-ml polystyrene jar (reservoir) with a screw cap, and a 20-mm section of galvanized water pipe as a counterbalance (Russell and Kay 1999). Before each sampling trial, the reservoir was filled to two-thirds capacity with clean water. The trap was then placed on the

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surface of the water and the weight of the metal collar around the trap caused the trap to invert and then float on the surface (Fig. 1). Immature mosquitoes were guided by the inverted funnel into the reservoir.

Mosquitoes: Immature *Ae. aegypti* were reared from eggs obtained from a colony maintained at the National Institute of Hygiene and Epidemiology. The colony was originally established in 1999 from larval *Ae. aegypti* collected from Ha Tay Province in northern Vietnam.

Sampling Ae. aegypti *from jars:* Laboratory evaluations of funnel traps for sampling immature *Ae. aegypti* were undertaken in 0.28-, 0.38-, 0.48-, and 0.52-m-diameter jars that were filled to 90% capacity with 30, 50, 100, and 150 liters of water, respectively. Ten replicate jars were each inoculated with 25, 50, 100, 200, 400, and 800 3rd and 4th instar *Ae. aegypti.* Larvae were acclimatized in the jars for 2 h and a funnel trap was then added to each jar. To determine whether the duration of funnel trap sampling had an effect on the numbers of larvae collected, 3 sampling periods were used (8, 16, and 24 h). The funnel traps were either placed in the jars at 0800 h and retrieved at 1600 h (8 h), or the traps placed in the jars at 1600 h and retrieved at either 0800 h (16 h) or 1600 h (24 h) the following day. After collection, the contents of each funnel trap were emptied into a white tray and the larvae were counted.

Data analysis: The proportion of larvae recaptured during each trial was calculated by dividing the number collected in the trap by the absolute number in the jar. The relationship between the proportion of larvae recaptured, jar size, and the duration of funnel trap sampling was analyzed by 2-way analysis of variance (ANOVA). Where necessary, an all-pairwise comparison test at $P < 0.05$ (Tukey 1953, cited in Zar 1999) was used to determine significant differences within each factor. Recapture rates were square root transformed before analysis. Mean recapture rates for each jar size and sampling period were calculated and were designated by $mean\ recapture\ rate_{i,j}$, where i represents jar size (0.28-, 0.38-, 0.48-, or 0.52-m diameter) and j represents the funnel trap sampling period (8, 16, or 24 h).

Error associated with funnel trap sampling from a single jar: To evaluate whether a calibration factor ($1/mean\ recapture\ rate_{i,j}$) and a single funnel trap sample could be used to estimate the total number of larval *Ae. aegypti* in a jar, we used the variation in funnel trap recapture rates to calculate the percentage error in the estimated number of larvae. For example, based on a calibration factor of $1/mean\ recapture\ rate_{0.28\ m,\ 8\ h}$ of 2.7 for funnel trap samples from a 0.28-m-diameter jar over an 8-h sampling period, the percentage error in the estimated number of mosquito larvae in each trial was calculated according to % error in estimate = $(recapture\ rate_{0.28\ m,\ 8\ h} \times 2.7 - 1) \times 100$. This calcu-

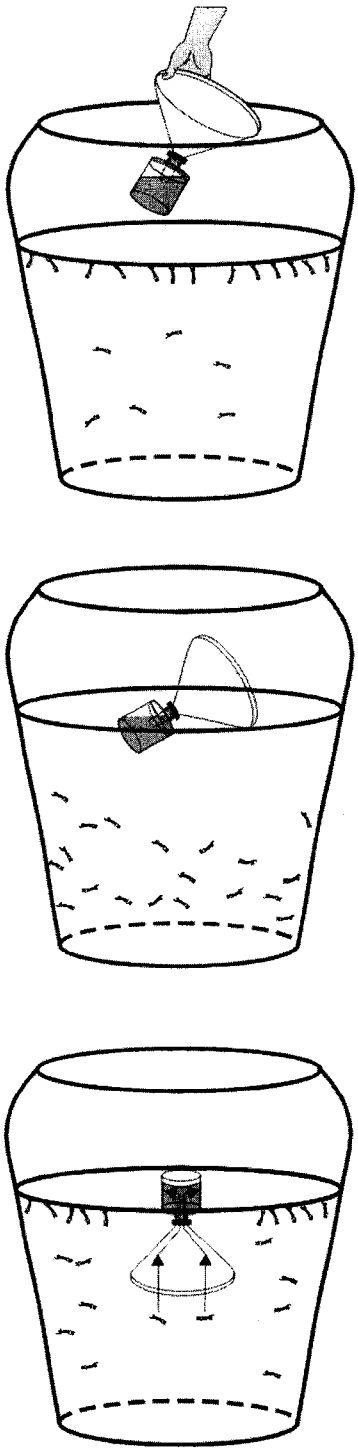

Fig. 1. Sampling of larval *Aedes aegypti* from water storage jars. The funnel trap was filled to two-thirds capacity with water and was placed on the surface of the water. The weight of the metal collar around the trap caused the trap to invert and then float on the surface. Immature mosquitoes were guided by the inverted funnel into the reservoir.

lation was repeated for each *recapture rate*$_{0.28\ m,\ 8\ h}$ to give a total of 60 error values and 5th, 25th, 75th, and 95th percentiles were calculated for these values. This was repeated for each jar size and funnel trap sampling period.

Error associated with funnel trap sampling from multiple jars: A computer simulation model was developed to describe the error associated with estimating the total number of larvae from multiple jars (Fig. 2). In this model, we generated n jars and assigned each jar a random number of larval *Ae. aegypti* drawn from a population of between 1 and 400 larvae per jar. Each jar then was assigned 100 randomly selected funnel trap *recapture rates*$_{i,j}$ based on data from the laboratory trials. The number of larvae in each jar was then multiplied by each *recapture rate*$_{i,j}$ to produce 100 simulated funnel trap catches. Each simulated funnel trap catch was then divided by the *mean recapture rate*$_{i,j}$ to produce an estimated number of larval *Ae. aegypti* in each jar. The total estimated number of mosquito larvae from n jars was then calculated for each simulation trial, and the percentage error for this total ([total estimated number − total actual number]/ total actual number × 100) was calculated. Based on simulations for 100 sets of n = 5, 10, 20, 50, and 100 jars, 5th, 25th, 75th, and 95th percentiles were calculated for the percentage error in total estimated larval numbers.

RESULTS

The funnel trap sampling method was 100% sensitive for detecting the presence of immature *Ae. aegypti* in 0.28- to 0.48-m-diameter jars with larval densities as low as 25 larvae per jar (lowest density tested). In the 0.52-m-diameter jars, funnel traps were 100% sensitive at larval densities of 25 per jar; however, at densities of 50 larvae per jar the sensitivity was 70, 80, and 90% at 8, 16, and 24 h, respectively. The decreased sensitivity of funnel trap sampling at 50 3rd and 4th instars per jar compared to 25 3rd and 4th instars per jar was unexpected, yet significant (Fisher exact test, P = 0.012). Funnel traps were 100% sensitive at densities ≥ 100 3rd and 4th instars per jar.

Analysis of recapture rates of immature *Ae. aegypti* from 0.28- to 0.52-m-diameter jars indicated that jar size and duration of trapping period each had a significant effect on funnel trap sampling efficiency (Fig 3). Jar size had the largest effect on the percentage recapture of immatures in funnel traps (ANOVA, $F_{3,707}$ = 166.3, $P < 0.001$). The overall mean recapture rate of 39.2% from 0.28-m-diameter jars was significantly greater ($P < 0.05$) than the 31.4%, 20.5%, and 13.4% recapture rates from 0.38-, 0.48-, and 0.52-m-diameter jars, respectively. The duration of funnel trap sampling had a smaller yet significant effect on the recapture rate (ANOVA, $F_{2,707}$ = 5.238, P = 0.006). Significantly ($P < 0.05$) fewer larvae were collected in funnel traps set for 8 h (mean recapture = 24.1%) than in traps set for 16 (27.3%) or 24 h (27.0%). Recapture rates in funnel traps set for 16 and 24 h were not significantly different ($P > 0.05$). The interaction term (jar size × duration of trapping) was not significant (ANOVA, $F_{6,707}$ = 1.1, P = 0.368), indicating that the effect of duration of trapping is independent of the size of the jar.

Based on the variability in the funnel trap recapture rates in the laboratory trials (Fig. 3) and calibration factors for each combination of jar size and trapping period equal to 1/*mean recapture rate*$_{i,j}$, the variability in the estimated numbers of immature *Ae. aegypti* was calculated (Fig 4). Because of the large variation in funnel trap recapture rates, a correspondingly large variation was found in the estimated numbers from a single jar. For example, for 0.28-m-diameter jars sampled over an 8-h period, 25% of funnel trap trials overestimated the number of larvae by more than 32%, and 25% of trials underestimated the number of larvae by more than 27%. The variation was greatest in estimates from 0.52-m-diameter jars; based on 8-h samples, 25% of trials overestimated the number of larvae by more than 57%, and 25% of trials underestimated the number of larvae by more than 70%.

Based on results from the computer simulation model, the variation in total estimated numbers of larvae decreased as the number of jars increased (Fig. 5). These results were based on pooled 16- and 24-h funnel trap data because no significant difference was found in mean funnel trap recapture rates for these periods (Fig. 3). If funnel traps were used to sample larvae from 5 0.28-m-diameter jars, then the total estimated number of larvae from these jars would be within −33.2% to +28.5% of actual population on 95% of occasions. In contrast, if funnel traps were used to sample larvae from 100 0.28-m-diameter jars, the total estimated number of larvae from these jars would be within −7.5% to +6.3% of actual population on 95% of occasions. This relationship between the overall error in estimated numbers and the number of jars surveyed was consistent for 50-, 100-, and 150-liter jars. However, the percentage error in estimated numbers was greater in the large jars (95% confidence interval for 100 0.52-m-diameter jars, −14.8% to +10.8%) compared with small jars (95% confidence interval for 100 0.28-m-diameter jars, −7.5% to +6.3%).

DISCUSSION

The heterogeneity in the distribution of containers positive for *Ae. aegypti* within communities usually means that a substantial number (≥100) of premises must be surveyed to provide accurate indices of risk (Breteau 1954), and estimates of the standing crop of immatures in a locality. For example, to determine the efficacy of control of *Ae. aegypti* in a community-based control program in

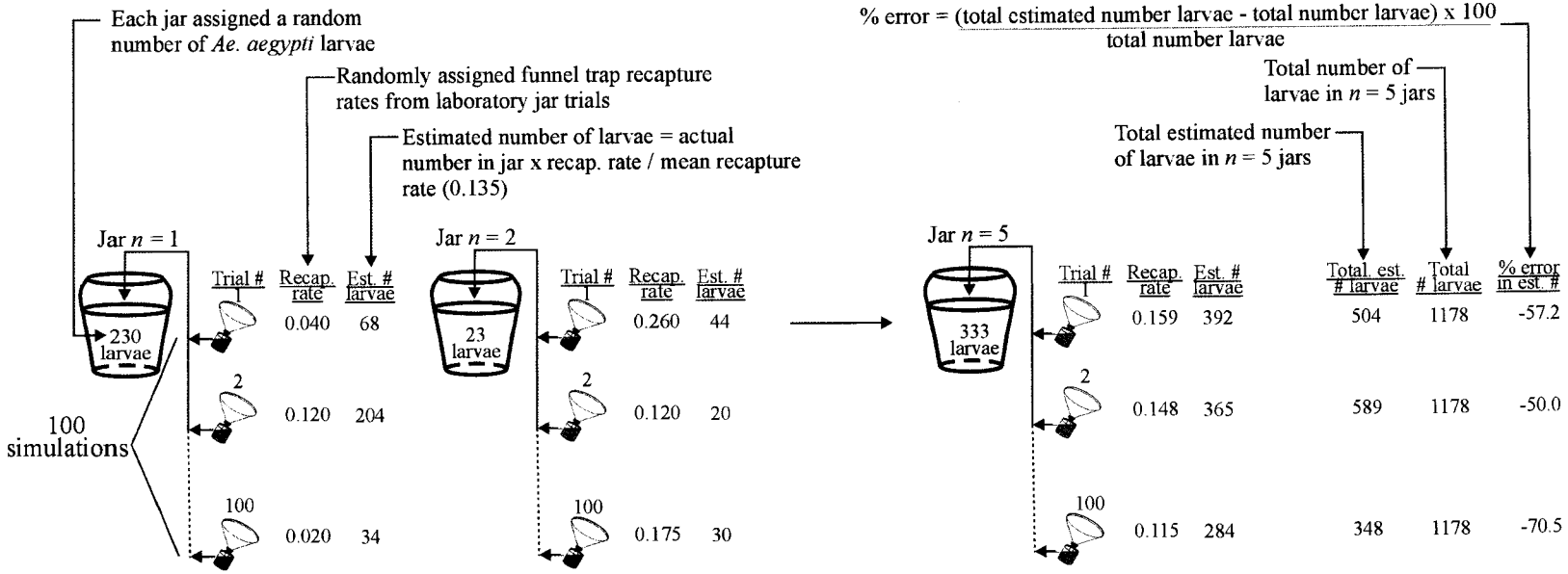

Fig. 2. Example of calculations in the computer simulation model to estimate the percentage error in the total number of larval *Aedes aegypti* in a series of 5 0.52-m-diameter jars, based on funnel trap sampling over a 16- to 24-h period and a *mean recapture rate*$_{0.52\ m,\ 16\text{–}24\ h}$ of 0.135. For each simulation, the actual number of larvae in each jar was multiplied by the randomly selected funnel trap recapture rate to produce a simulated funnel trap catch. Each simulated funnel trap catch was then divided by the *mean recapture rate*$_{0.52\ m,\ 16\text{–}24\ h}$ (0.135) to produce an estimated number of larval *Ae. aegypti* in each jar. The total estimated number of mosquito larvae from the 5 jars was calculated for each trial, and the percentage error for this total was calculated.

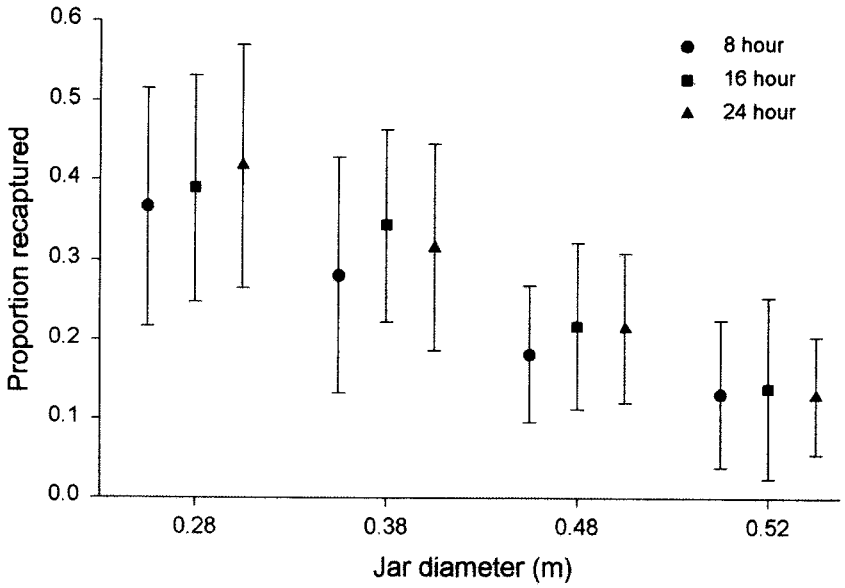

Fig. 3. Mean ± SD recapture rates for *Aedes aegypti* from jars with funnel traps (based on larval populations of 25, 50, 100, 200, 400, and 800 per jar and 10 replicates for each larval density.

Vietnam (Kay et al. 2002), 100 houses were randomly selected and inspected every 3 months. The frequency of each container category and its productivity in terms of 3rd and 4th instar of *Ae. aegypti* and *Ae. albopictus* (Skuse) were determined and control efficacy was expressed as the percentage reduction in the total numbers of 3rd and 4th instars, compared to pretreatment levels. This requires the direct counting of immatures from small containers, including water storage jars, and often it is physically difficult for survey staff to sieve the contents of jars >50 liters in capacity, or undesirable from the resident's point of view because of water loss or increased turbidity. The use of funnel

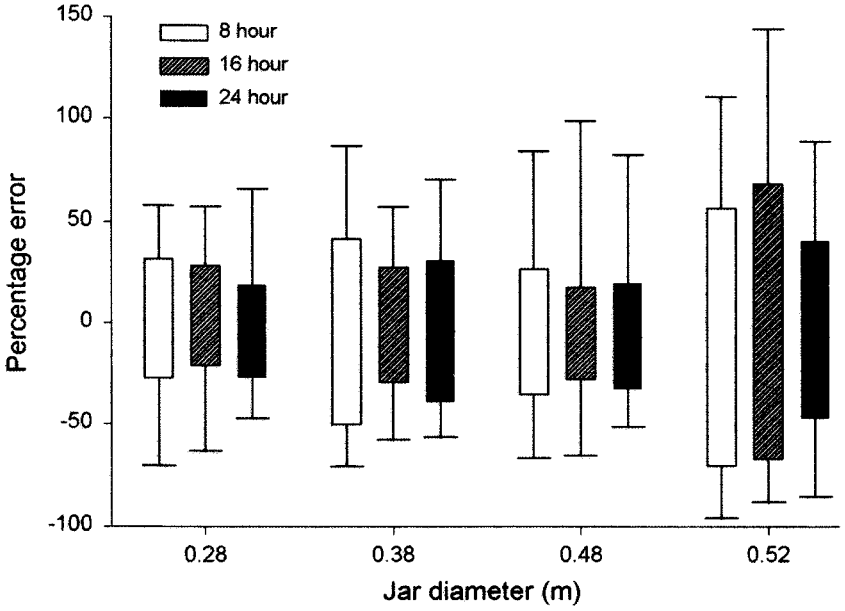

Fig. 4. Box plots of percentage error in estimated numbers of larval *Aedes aegypti* in a single jar based on a calibration factor of $1/mean\ recapture\ rate_{i,j}$ and 60 laboratory trials with funnel traps (lines, 5th and 95th percentiles; bars, 25th and 75th percentiles).

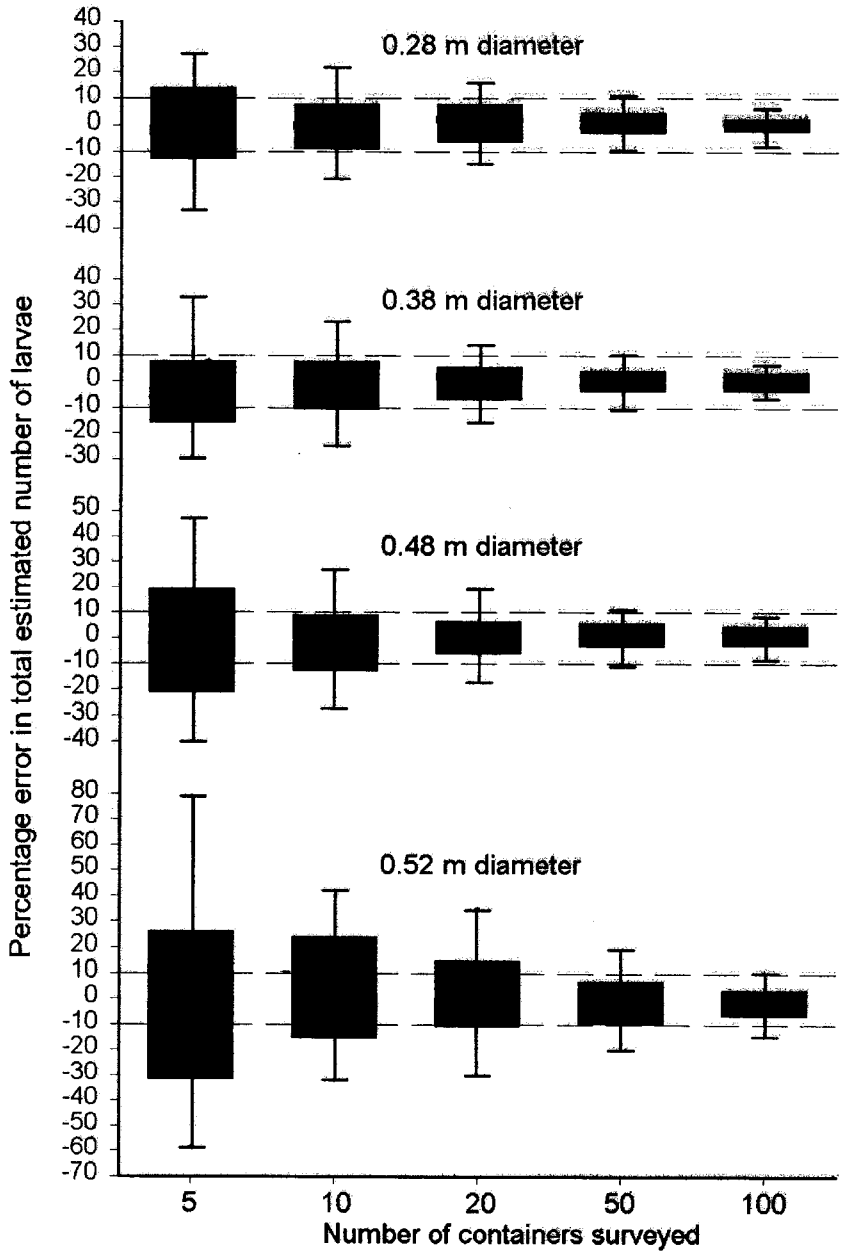

Fig. 5. Box plots of percentage error in total estimated numbers of 3rd and 4th instars of *Aedes aegypti* in 0.28-, 0.38-, 0.48-, and 0.52-m-diameter jars as a function of number of jars surveyed. Calculations were based on 100 computer simulations with combined 16- and 24-h funnel trap recapture data. Numbers of larval *Ae. aegypti* in the jars were selected from a randomly distributed population ranging between 1 and 400 larvae per jar (lines, 5th and 95 percentiles; bars, 25th and 75th percentiles).

traps to sample immatures from water storage jars is a passive method of sampling that does not require lifting of heavy containers by field personnel and does not affect water quality.

The evaluation of funnel traps for estimating numbers of *Ae. aegypti* was based on calibration factors specific for each jar size. Usually funnel traps are set during the late afternoon and recovered the next morning because they are less likely to suffer interference during daily water gathering (Kay et al. 1992). Because no significant difference was found between mean recapture rates for 16- and 24-h sampling periods, calibrations were based solely on jar size, that is, 2.5, 3.0, 4.6, and 7.4 for

0.28-, 0.38-, 0.48-, and 0.52-m-diameter jars, respectively. Under field conditions, an assessment of jar size would have to be made and an appropriate calibration factor used to calculate the total number of larvae in the container. This would mean field staff could set funnel traps during the afternoon one day and retrieve them the following morning, sum the funnel trap counts for each jar size and apply the appropriate calibration factor, and then do a summation for total abundance.

The calibration factors for each of the 4 jar sizes were based on trials conducted in jars with constant water depths. Under field conditions, water levels in jars may vary considerably and this may have an effect on funnel trap sampling. Obviously, the depth of the funnel trap itself (180 mm) is an impediment to sampling jars with water levels less than 200 mm. Additional laboratory experiments involving a range of different water depths and jar shapes could be conducted; however, we would argue whether a complex calibration factor based on a combination of jar shape, diameter, and water depth would be practical under field conditions. We plan to undertake a field evaluation in which funnel trap estimates of larval abundance will be compared to absolute numbers determined by sieving and direct counting. From these investigations, we will be able to determine the sensitivity and error associated with use of funnel traps and a set of calibration factors based on jar diameter only, to estimate numbers of 3rd and 4th instars under field conditions.

Based on our laboratory data, our computer simulation results demonstrate that the estimated numbers of immatures are reasonably accurate if a sufficient number of positive jars are surveyed (95% confidence interval for 50 0.52-m-diameter jars, −19.9% to +17.8%), and it is not uncommon to find infestation rates for *Ae. aegypti* of this order. For example, in central Lao People's Democratic Republic, Tsuda et al. (2002) surveyed 136 houses and found 218 (57%) medium- to large-sized jars that were positive for immature *Ae. aegypti.* In a survey of 3,000 houses in Cambodia, Chantha et al. (2001) found 2,069 (85%) water storage containers that were positive. In these situations, funnel trapping would provide an accurate estimate of the total number of immatures.

These results need to be compared with those from dipping and netting. Funnel traps are more sensitive than dipping for detecting immature *Ae. aegypti.* Although the dipping evaluations were completed in 200-liter drums, slightly larger than the jars used in our trials, Tun-Lin et al. (1994) found that dipping was an insensitive method for detecting immature *Ae. aegypti,* with only 73% and 11% of samples positive for 3rd and 4th instars, respectively, at densities of 100 larvae per drum. In contrast, 90% of funnel trap samples from 0.52-m-diameter (150-liter) jars with 25–50 larvae per jar were positive. The large variation in percentage recovery obtained with dipping indicated the unreliability of this method as a quantitative tool (Tun-Lin et al. 1994).

Nets are routinely used in our programs (Nam et al. 1998, Kay et al. 2002) for sampling water storage containers because they are superior to dipping in terms of both sensitivity and as a quantitative method to estimate absolute numbers (Tun-Lin et al. 1994), and also as a method for sampling copepods and other predators (Nam et al. 2000). A mean ± SD recovery rate of 25.7 ± 6.5% was obtained when using nets to sample 4th instars from 200-liter drums containing 132 liters of water (Tun-Lin et al. 1994). This would equate to a calibration factor of 3.9 to convert the number collected in the net to an absolute number in the drum. Therefore, based on a single sample, we calculated that 95% of estimates would be within −44% and +33% of the true population. This is better than what we obtained when using funnel traps to sample 0.52-m-diameter jars (95% confidence interval, −95 to +138%). Therefore, on this basis, it seems that nets provide a better estimate than funnel traps of the numbers of *Ae. aegypti* in water storage jars. However, the efficacy of net sampling depends on the skill and attentiveness of the collectors and netting may be unacceptable in some situations where sediment is disturbed from the bottom of the jar.

Because of the need to determine the number of immature *Ae. aegypti* in individual containers, we require reliable sampling methods that are suitable for use under a variety of field conditions. It is unlikely that any 1 method will be suitable for sampling every type of habitat of *Ae. aegypti* and funnel traps could be used as a quantitative survey tool where necessary. However, for small water storage jars ≤50 liters in capacity, we would still recommend direct counts of immatures by sieving the entire contents of the jar through a net. For medium (≥50 liters) and large water storage containers, we recommend the use of nets with an appropriate calibration factor (Tun-Lin et al. 1994), or where necessary, funnel traps. For sampling immatures from subterranean habitats such as wells and service manholes (Russell and Kay 1999), funnel traps are especially applicable. In regions such as northern Thailand and southern Vietnam, jar sizes can reach capacities of ≥2,000 liters, and separate calibrations will be required.

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