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QUANTITATIVE SAMPLING OF IMMATURE *AEDES AEGYPTI* IN METAL DRUMS USING SWEEP NET AND DIPPING METHODS

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ABSTRACT. The behavior of aquatic stages of *Aedes aegypti* was evaluated in 200-liter metal drums to develop improved surveillance. When known numbers of larvae recovered with a rectangular net and with a standard dipper were compared, all stages except 1st instar were most numerous in the top ⅓ of the drum. Water temperature was the only significant variable affecting the vertical distribution of 4th-instar larvae; light intensity and pH were nonsignificant factors. Rust in the water was also thought to influence distribution towards the surface layer. When 100–400 immatures were released into a full drum, immatures were detected by sweeping and dipping the surface layer on 93.3 and 72.9% of trials, respectively. The correlation coefficients (r) of recoveries of known numbers of immatures by sweeping and dipping varied from 0.92 to 0.98 and from 0.38 to 0.89, respectively. The coefficients of variation were 2–5 times smaller for sweeping than for dipping. Recoveries from dipping were shown to be affected by the water volume in the drum. Counts of 4th-instar larvae from one sweep around the top are sufficient to assess productivity in drums.

INTRODUCTION

Although zoologists regularly use various forms of nets for collecting aquatic organisms, medical entomologists have adopted various shapes and sizes of ladles or dippers for sampling immature mosquitoes (Russell and Baisas 1935, Russell et al. 1963, Hagstrum 1971, Wada et al. 1971). Service (1976) reviewed many of these and concluded that dipping is often biased for particular species and even instars. In counting immatures from a dipper using a pipette, Earle (1956) reported that even experienced collectors overlooked many of the younger instars and even a small percentage of 3rd- and 4th-instar and pupal stages of mixed *Anopheles, Culex,* and *Aedes.*

Current larval indices (Breteau 1954, Sheppard et al. 1969, World Health Organization 1986) used to monitor *Aedes aegypti* (Linn.) populations are indicators of prevalence and as such, bear little relationship to adult female abundance and risk of dengue transmission (Kay et al. 1987, Reiter 1992). In order to improve these indices, we assumed *a priori* that productivity of containers should be considered. Whereas numbers in small containers (e.g., flower vases, ant traps, bottles, tin cans) can be counted directly, those in large containers (e.g., tanks, wells, drums) cannot.

As 200-liter drums are a major breeding site for *Ae. aegypti,* we compared quantitative sampling efficacy by dipper and net. During this comparison, questions arose regarding stratification of immatures in the water column; the effect of different water volumes, temperature, and water quality; the number of dips or sweeps required to provide an estimate of productivity; and the immature stage that could be measured most reliably. This paper addresses these issues with the overall objective of providing a method to facilitate quantitative sampling of immature *Ae. aegypti* to improve larval surveillance.

MATERIALS AND METHODS

Sampling apparatus: The white cotton net (100-μm gauze) was 20 cm long and rounded at the base. It was fitted to a 10-cm-wide × 20-cm-high aluminum frame with a 180-cm-long extendable handle. This shape maximized sampling around the perimeter of the drums. The seam of the net was on the outside to minimize trapping and injuring immatures.

The net was immersed gently against the side of the 200-liter drum and swept once around the perimeter (diam = 57 cm) in less than 10 sec. The net sampled a water volume of 29.5 liters. When sampling below the surface, the net was rotated sideways on completion of the sweeping procedure and brought rapidly to the water surface to prevent the collection of additional immatures from above.

The metal dipper, 14 cm in diameter and holding 350 ml of water, was mounted on a 35-cm-long handle. The dipper was directed at aggre-

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Table 1. Percentage recovery of 200 introduced 4th-stage *Aedes aegypti* larvae, when sampled from the top of a 200-liter metal drum, containing different water volumes (laboratory data 1988).

Water volume (liter)	Mean no. caught ± SD[1]		% recovery ± SD	
	Sweep	Dip	Sweep	Dip
200	52.2 ± 12.7	5.4 ± 3.1	26.1 ± 6.3	2.7 ± 1.5
132	51.3 ± 12.9	2.5 ± 1.8	25.7 ± 6.5	1.3 ± 0.9
67	58.5 ± 13.6	0.2 ± 0.4	29.3 ± 6.8	0.1 ± 0.2

[1] Based on 5 samples for each sweep and dip.

gates of *Ae. aegypti* larvae when the drum was full (200 liters) or ⅔ full (133 liters) because this is standard sampling practice. When the drum was only ⅓ full (67 liters), it was difficult to see aggregations of *Ae. aegypti* larvae and so dipping was at random.

From 1988 to 1990, experiments were performed using known numbers of immatures introduced into 200-liter drums in the Queensland Institute of Medical Research insectary (28 ± 1°C) and at Townsville and Charters Towers, northern Queensland. The same set of immatures was used for each replicate, except for those which were sluggish or obviously damaged.

Sweeping and dipping at different water levels: In the laboratory, 200 4th-instar *Ae. aegpyti* were introduced into a 200-liter drum when it was ⅓, ⅔, and full of water. At each water level in each separate experiment, 10 sweeps and 10 dips were taken from the surface. Sweeping and dipping were performed alternately at intervals of 5 min. The numbers of 4th-stage larvae collected per sweep and per dip were counted and the percentage recovery determined.

Sweeping and dipping from the top at different immature densities: The percentage recoveries of *Ae. aegypti* larval and pupal stages were compared by sweeping and dipping with 200, 400, 600, 800, and 1,000 of each larval stage per drum and for up to 600 for pupae. Ten sweeps and 10 dips from the top of a full drum were done at intervals of 5 min or greater. The numbers of positive recoveries from the 2 methods was also recorded for densities of 100–400 immatures.

These laboratory experiments were repeated in the field for selected immature stages during 1989 and 1990. Because the net sampled a water volume of 29.5 liters and the dipper only 0.35 liter, numbers collected were expressed as a mean percentage recovered per liter and Pearson's correlations calculated against known numbers present in the drum. Coefficients of variation were estimated for gauging the reliability of both methods.

Stratification of immatures: Two laboratory and 2 field (Townsville and Charters Towers) experiments were carried out by introducing 100 and 200 individuals of all stages into a full 200-liter drum. Each combination, as outlined above, was evaluated separately. Five replicates, at 5-min intervals, were taken by sweeping at each level (top, middle, and bottom) in 2 ways:

1) Net sampling starting at the top and continuing to the middle and bottom level as the first set in a series of 5. As before, immatures were counted and undamaged ones returned for the next sample. Numbers were made up with new stock.
2) Samples were completed at the top before proceeding to sample, in order, middle and bottom. These data were evaluated by ANOVA.

Number of replicate sweeps required for quantitative assessment: The percentage larval recovery was compared for each successive replicate sweep. This was done in Townsville (1989) and at Charters Towers (1990). Recoveries from

Table 2. Relative sensitivity of sweeping and dipping *Aedes aegypti,* based on number of positive recoveries from a full drum (200 liters) with 100–400 immatures released.

No. released	Number of positives, sweeping : dipping (no. of trials) with different stages[1]				
	I	II	III	IV	P
100	10:4 (10)	10:7 (10)	15:11 (15)	20:4 (35)	15:13 (15)
200	10:6 (10)	10:8 (10)	15:14 (15)	35:34 (35)	15 13 (15)
400	10:6 (10)	10:10 (10)	15:15 (15)	5:4 (5)	15:15 (15)

[1] I, II, III, IV = 1st-, 2nd-, 3rd-, and 4th-instar larvae, respectively; P = pupae.

Table 3. Pearson's correlations (and coefficients of variation ± SD) for numbers of immature *Aedes aegypti* recovered by sweeping compared to dipping, in relation to absolute numbers of larvae (100–1,000) or pupae (100–600) introduced.

Immature stage	Laboratory (1988)		Townsville (1989)	
	Sweep	Dip	Sweep	Dip
I	0.95 (25.5 ± 15.5)	0.73 (77.7 ± 38.1)		
II	0.95 (21.8 ± 14.1)	0.59 (52.3 ± 22.0)		
III	0.96 (11.7 ± 5.9)	0.80 (43.5 ± 13.8)	0.96 (16.8 ± 6.5)	0.78 (60.7 ± 27.1)
IV	0.98 (16.3 ± 8.3)	0.43[a] (78.3 ± 56.3)	0.96 (17.7 ± 5.1)	0.73 (53.2 ± 15.5)
Pupae	0.92 (21.0 ± 22.0)	0.48[a] (51.3 ± 17.7)	0.94 (15.5 ± 5.9)	0.38[b] (68.0 ± 24.4)

Levels of significance $P \leq 0.0001$ except where designated a = $P < 0.01$ or b = nonsignificant.

the first sweep were correlated with known numbers introduced (100–1,000) and compared with correlation coefficients based on mean number collected from 5 sweeps.

Environmental factors affecting recovery: Temperature, pH, light intensity, and presence of rust floculate were recorded for all experiments. The independent and joint effects of the first 3 factors were analyzed by multiple linear regression (SAS Institute 1988). As rust was scored only as positive or negative, it was not included in this analysis.

RESULTS

Effect of different water volumes in drum: When 200 4th-instar larvae were introduced (Table 1) into a drum with 67, 133, or 200 liters of water, percentage recovery per sweep was similar for all volumes, ranging from 25.7 to 29.3% ($F = 0.9$; df = 2,27; $P = 0.42$). This was not the case with dipping where the percentage recovery was decreased as the water level was reduced (e.g., 2.7 ± 1.5%, 1.3 ± 0.9%, and 0.1 ± 0.2% [$F = 15.7$; df = 2,27; $P < 0.0001$]). With 200 4th-instar larvae in 67 and 133 liters respectively, positive dips were recorded on 20 and 80% of 30 trials.

Recoveries by sweeping and dipping in relation to numbers and stages present: From 225 trials involving release of 100–400 immatures of all stages, at least one immature was detected by sweeping and dipping the surface of a full 200-liter drum on 93.3 and 72.9% of occasions, respectively (Table 2). With 100 immatures present, sweeping was approximately twice as sensitive as a dipper.

When surface sweeping was compared to dipping in 200-liter drums in both laboratory and field studies, larger numbers of *Ae. aegypti* of all stages were recovered by sweeping. When this was adjusted to mean percentage recovery per liter of water sampled, the dipper actually gave higher returns because of the biased nature of surface sampling with this method.

The percentage recovery/liter for 1st and 2nd instars was generally less than for later stages. This trend was consistent for both dipping and sweeping (see Table 4), and for laboratory and field studies. Detailed results are presented in Tun-Lin (1992[3]).

Pearson's correlations (Table 3) for recovery rates of known numbers of larvae introduced by sweeping and dipping in the drums ranged from 0.92 to 0.98 ($P < 0.0001$) and from 0.38 ($P > 0.05$) to 0.89 ($P < 0.0001$), respectively. Calculation of the coefficients of variation ± SD on mean recovery of *Ae. aegypti* immatures indicated that the variation by dipping was 2–5 times higher than by sweeping for all aquatic stages.

Stratification of immatures: In an initial test involving the release of 200 4th-stage *Ae. aegypti,* mean percentage recoveries per sweep from top, middle, and bottom strata of a full drum were 26.1 ± 6.3, 5.0 ± 1.2, and 3.5 ± 1.1%, respectively, indicating larval stratification in the water column ($F = 111.9$; df = 2,27; $P < 0.0001$). In a drum with 133 liters, mean percentage recoveries per sweep for top and bottom samples were 25.7 ± 6.5 and 7.0 ± 3.0% respectively ($t = 8.3$, $P < 0.0001$).

Data for all aquatic stages in the laboratory (see Tun-Lin 1992[3]) and at Townsville and Charters Towers in 1989–90 are combined in Table 4. The percentage recovery of all stages, except perhaps for 1st instars, was greatest from the top stratum. Results were similar, regardless of the order of the sweeping procedure.

When all data of recovery by sweeping of all

[3] Tun-Lin, W. 1992. Studies on the ecology and biology of *Aedes* (*Stegomyia*) *aegypti* (Linnaeus) (Diptera: Culicidae) immatures in Queensland, with special reference to improved surveillance. Ph.D. dissertation. Faculty of Medicine, University of Queensland, Brisbane, Australia.

Table 3. Extended.

Townsville (1990)	
Sweep	Dip
0.94 (23.0 ± 5.2)	0.85 (79.7 ± 72.7)
0.95 (17.5 ± 5.4)	0.89 (51.0 ± 38.4)

aquatic stages were combined for ANOVA, aquatic stage ($F = 37.6$, $df = 4$, $P < 0.0001$), stratum ($F = 436.9$, $df = 2$, $P < 0.0001$), numbers introduced ($F = 13.0$, $df = 1$, $P = 0.0003$), and year of experiment ($F = 3.3$, $df = 2$, $P < 0.04$) significantly affected recoveries. All interactions were also significant ($P < 0.0001$), indicating many inconsistencies.

The significance levels of strata, number introduced, year, and their interactions resulting from separate ANOVAs of percent recovery for each instar are shown (Table 5). Numbers collected from each stratum were highly significant ($P < 0.0001$) for all stages. Sampling from the top produced the highest percent recovery for all but 1st stages, which were usually more numerous at the bottom ($P < 0.01$).

Differences in percentage recovery of 3rd- and 4th-instar larvae in relation to numbers introduced were nonsignificant. There were significant differences ($P < 0.05$ to <0.0001) for all other stages including pupae, indicating vagaries between the numbers sampled and absolute numbers.

Fourth-stage percent recovery differed less by year ($P < 0.05$) when compared to other immature stages where the significance levels varied from $P < 0.01$ to $P < 0.0001$. The water conditions were different in each year (see later).

Lack of significant interactions indicates reliability of the sampling method. However, all stages had at least one significant interaction. For 4th instars, recoveries were not consistent for Numbers introduced × Year and for pupae, Strata × Year was significant. Third-instar larvae showed 2 highly significant interactions whereas 1st and 2nd instars were significant for all 3. On this basis, it was decided that 4th instars were not only the most easily recovered but also gave the most reliable results in relation to a range of 100–1,000 immatures per drum.

Number of sweeps for quantitative assessment: When numbers of 4th-instar larvae caught in the first sweep of the surface of the drum were correlated with known numbers of 100–1,000 present, Pearson's correlations for 1989 and 1990 field data were $r = 0.99$ and 0.98, respectively. These correlations were higher than when correlations were based on mean numbers collected from 5 sweeps, separated by 5-min intervals, *viz.*, $r = 0.96$ and 0.95. In 1989, the water in the drum was 28°C and discolored by rust whereas in 1990, the water was clear and averaged 33°C.

Environmental factors influencing collection: When multiple regression was applied to determine the influence of temperature (10.5–33°C), light intensity (5,500–57,750 lux), and pH (7.0–7.8) on numbers of 4th instars recovered by sweeping, temperature was the only significant variable in both type 1 ($F = 51.3$, $df = 1$, $P = 0.002$) and type 3 models ($F = 14.5$, $df = 1$, $P = 0.02$). The influence of rust could not be examined in the above analysis but it was observed

Table 4. Mean percentage recovery/sweep of *Aedes aegypti* immatures at 3 different levels in a 200 liter metal drum full of water (number of replicates = 15), field and laboratory results combined for 1988–90.

No. of each immature stage introduced	Sampling stratum	Mean % recovery/sweep ± SD by stage[1]				
		I	II	III	IV	P
100	Top	2.9 ± 2.5	5.7 ± 3.9	16.3 ± 6.1	21.0 ± 8.6	17.6 ± 6.6
	Middle	3.3 ± 3.0	4.2 ± 3.1	4.9 ± 2.6	6.4 ± 3.0	3.9 ± 1.3
	Bottom	4.4 ± 3.1	2.9 ± 2.9	3.2 ± 2.1	4.0 ± 3.1	1.9 ± 1.6
200	Top	10.4 ± 7.4	11.8 ± 6.8	19.4 ± 9.25	23.3 ± 8.1	15.9 ± 4.5
	Middle	5.1 ± 3.3	3.7 ± 1.5	4.8 ± 1.7	5.6 ± 1.8	1.9 ± 1.3
	Bottom	6.0 ± 2.6	4.1 ± 2.7	3.8 ± 1.6	3.9 ± 1.6	1.0 ± 0.9

[1] I, II, III, IV = 1st-, 2nd-, 3rd-, and 4th-instar larvae, respectively; P = pupae.

Table 5. Significance of various factors and their interactions resulting from ANOVAs of percentage recovery of each *Aedes aegypti* immature stage by sweeping at different levels from 200-liter drums, 1988–90.

Source of variation	df	Significance levels at stage, $P <$[1]				
		I	II	III	IV	P
Strata	2	0.01	0.0001	0.0001	0.0001	0.0001
Number introduced	1	0.01	0.0001	NS[2]	NS	0.05
Year	2	0.001	0.0001	0.01	0.05	0.01
Strata × Number introduced	2	0.0001	0.0001	NS	NS	NS
Strata × Year	4	0.05	0.0001	0.0001	NS	0.01
Number introduced × Year	2	0.0001	0.0001	0.0001	0.0001	NS

[1] I, II, III, IV = 1st-, 2nd-, 3rd-, and 4th-instar larvae, respectively; P = pupae.
[2] NS = not significant.

that its presence changed larval distribution by forcing more larvae to the top layer.

The regression for numbers of 4th instars recovered from the first sweep in relation to water temperatures from 10.5 to 33.0°C equalled 26.2–0.37T ($r^2 = 0.8$, $n = 8$). Comparison of numbers recovered from the first sweep (Observed) with those from sweeps 2–5 (Expected) indicated significantly reduced catches for water temperatures of 10.5 ($\chi^2 = 113.4$, df = 1, $P < 0.0001$), 13 ($\chi^2 = 171.2$, df = 1, $P < 0.0001$), and 23°C ($\chi^2 = 9.4$, df = 1, $P < 0.01$), with that for 21.5°C ($\chi^2 = 3.1$, df = 1) bordering on significance. Above 23°C, the χ^2 analyzed for 5 successive sweeps were nonsignificant, indicating that 5-min intervals between sweeps at lower water temperatures were insufficient to allow the larvae to resurface.

DISCUSSION

Our findings demonstrate unequivocally that *Ae. aegypti* immatures can be sampled more effectively using a 20 × 10 × 20-cm net of 100-μm cotton gauze than with a 19-cm diam dipper. As one sweep of the net sampled a volume of almost 30 liters compared to 0.35 liter with the ladle, this method is more effective in detecting low numbers of immatures for general premise surveys, in order to calculate Breteau indices or in posttreatment follow-ups.

For quantitative estimation of *Ae. aegypti* productivity in 200-liter drums, recoveries by sweeping were not significantly affected by water level. This was not the case for dipping, as sampling was generally directed at aggregates of immatures visible on the water surface. When the water is not clear or is at a low level, however, dipping may become random thus reducing its efficacy. Percentage recovery by dipping the surface of a full drum compared to one containing only 67 liters fell from 2.7 to 0.1% of the numbers present. Under the same conditions, sweeping recovered from 26 to 29%.

When known numbers from 100 to 1,000 immatures of all stages were introduced into full drums in both the laboratory and the field, the relationship between sweeping and absolute numbers was superior to that for dipping. For dipping, the coefficients of variation were 2–5 times larger, indicating the unreliability of this method as a quantitative tool.

Our analyses indicate that assessment of productivity requires selection of a particular stage. Numbers of adults eclosing could theoretically be estimated from the product of numbers of the immature stage, and its survival rate to adulthood. However, different water quality, nutrient levels, and turnover rates of the water itself make this difficult to generalize. Hence, the most convenient stages to monitor would be the most advanced ones. Individual ANOVAs on recoveries of each stage demonstrated that behavior of immatures in the water column was related to recovery rates. It also demonstrated difficulties in quantitative estimations based on 1st and 2nd instars. Percent recovery rates in relation to numbers present were only consistent with 3rd and 4th instars. As more 4th instars were reliably collected compared to any other stage, and because they were more easily seen and counted, we elected to base our quantitative estimates on this stage. In New Orleans, Focks et al. (1981) estimated the standing crop of adult female *Ae. aegypti* by direct counts of pupae in containers from 0.5 to 11.9 liters. In drums, however, the percentage recovery varied with the numbers of pupae introduced, so we considered counts of pupae unsuitable as a basis for estimates of abundance.

The distribution of all but 1st and, to a lesser extent, 2nd instars, was strongly biased towards the top 20 cm of water. This is not surprising as young *Ae. aegypti* have been shown to survive

submerged for 3–5 h (Mitchell 1907) and that newly hatched larvae can live without air entering their tracheae for several days (Wigglesworth 1938). Reduced recovery rates of these early instars even from the bottom of the drum were influenced by their small size and the ease with which they were translocated into a central vortex created by sweeping. More efficacious sampling of these stages can be done by creating a vortex and sweeping through it at the bottom of the drum. However, such a method is unlikely to be popular with residents as it clouds the water with rust, leaves, and other debris, rendering it temporarily unsuitable for use.

Immatures are sensitive to vibration, to other mechanical stimuli, and to sudden changes in light intensity. Thus it is important that sweeping be accomplished with minimal disturbance. However, after the first sample had been taken, the majority of immatures ascended within the 5-min interval used between replicates. Although this was the case for 4th-instar larvae in water temperatures of 28–33°C, lowered metabolic activity allowed them to remain submerged longer in waters of 10–23°C. Consequently, in cooler temperatures, returns from sweeps subsequent to the first did not always reflect absolute abundance. On the basis of this and other field recoveries in Townsville (1989) and Charters Towers (1990), one sweep of the top stratum gave higher positive correlations ($r = 0.98$–0.99) with known numbers of 4th-instar *Ae. aegypti* than by taking a mean of 5 sweeps for each estimate (with 5-min intervals). A single sweep is therefore both more precise and cost effective for estimating numbers of 4th instars.

Water temperature was the only significant factor influencing recoveries of 4th-instar *Ae. aegypti* per sweep. It applies only to 4th instars because the recoveries of others, especially 1st and 2nd stages, were significantly affected by a number of factors. Furthermore, Bar-Zeev (1957) demonstrated that the aggregation behavior in response to temperature of early vs. late instars is different.

At Townsville, Charters Towers, and the other towns evaluated by Tun-Lin (1992[3]), the majority of positive containers were found in shade or semishade. Fourth instars tend to aggregate at the surface on the side shaded by the rim of the drums. When in full sunlight (noon), the larvae were more homogeneously distributed around the perimeter. Because sweeping covered the entire circumference, light intensity did not significantly affect recovery rates. As rainfall and tapwater, at approximately neutral pH, were the major water sources for the drums evaluated, this variable was also expected to prove nonsignificant.

Field surveillance techniques should be simple, cost-effective, and readily accepted by the community. Sweeping of the surface perimeter fulfils these requirements: 1) it causes minimal clouding of the water, 2) sweeping takes no longer than dipping, and 3) the net is inexpensive and therefore affordable in developing countries. One sweep of the surface offers improved sensitivity over dipping for detection of all aquatic stages of *Ae. aegypti*. Furthermore, it facilitates easy estimation of absolute abundance, based on 4th-instar larvae, which may form a basis for improved surveillance and prediction of adult numbers. The change from indices of prevalence to those of abundance is essential to achieving this goal.

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