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COMPARISON OF SAMPLING EFFICACY OF SWEEPING AND DIPPING FOR *AEDES AEGYPTI* LARVAE IN TIRES

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ABSTRACT. A sweep net with a flexible plastic frame was compared to a dipper for sampling efficacy for *Aedes aegypti* larvae in different-sized tires. When known numbers of mosquito larvae were introduced into tires, from 2 to 6.8 times more larvae were recovered by sweeping than by dipping. Sediment in the tires significantly reduced the recovery of larvae by both methods. Sampling efficacy was affected by water level and tire size. In relation to dipping, sweeping showed less variation in percentage recovery, higher sensitivity for detecting larvae and had better correlations with known numbers of larvae in tires. We conclude that sweeping is superior to dipping for sampling mosquito larvae in tires.

INTRODUCTION

Discarded tires constitute an important breeding habitat for mosquitoes, especially *Aedes aegypti* (Linn.) and *Aedes albopictus* (Skuse). In Puerto Rico and Miami, respectively, scrap tires accounted for <15% and 80% of containers harboring *Ae. aegypti* (Frank 1981) whereas the affinity of *Ae. albopictus* for this habitat is well known (Hawley 1988).

Halstead (1992) considered that *Ae. aegypti* control had "a glorious past but a dismal present". This breakdown in control is partly due to political, financial and social reasons, and we would contend that it is also due to overreliance on outdated and inappropriate surveillance tools. For example, the Breteau index (Breteau 1954) is an indicator of prevalence rather than abundance and yet, certain levels are used to infer safety or risk of dengue transmission (World Health Organization 1972). Implicit in the development of better methods for both surveillance and control is consideration of breeding site productivity.

The dipper is undoubtedly the most commonly used tool for sampling immature mosquitoes. Service (1976) reviewed different types and their inherent bias with respect to collection of different instars or species. The water inside the casing can be siphoned (Trpis 1972) or shaken out into a tray or individual larvae picked out. These procedures are often laborious and may not reflect the relative importance of the container type in terms of *Aedes* production.

The plankton net is a standard zoological tool for sampling aquatic organisms. We routinely use a 200 × 100 mm net of 100-μm mesh for detecting predacious cyclopids and mosquito immatures in tanks, drums and other large containers (Brown et al. 1992). Nets are particularly useful for sampling large habitats, especially when immature populations are low (Heathcote 1970, Service 1976).

In this paper, we describe a net with a flexible plastic frame that can accommodate different sized tires. We compare the practicalities and recovery rates of this net with a dipper with respect to detection of larvae and estimation of absolute abundance.

MATERIALS AND METHODS

Sampling devices: The 100-μm-mesh net was mounted on a flexible polyvinylchloride frame with a circumference of 63 cm and attached to a short handle (Fig. 1). The frame is constructed with 2 interlocking cable ties (Wattmaster P/L, Sydney). The ties, 548 × 12 (ribbed width 8 mm) × 2 mm, were threaded together via the top clasp and looped together so that the bottom half of the loop was of 2 thicknesses of cable tie. Each end was then fixed to its partner halfway up its length with a press stud. The shaft of the loop was then fitted with either a press stud or a leather loop that was used to adjust net circumference. The seam of the net was kept on the outside so that mosquito larvae would not be trapped in the seam or be injured by it. Because of the flexibility of the frame, the volume sampled depends on the dimensions of the tire and also on the volume of water therein.

A 14-cm-diam metal dipper holding 350 ml of water was fixed to a wooden handle 35 cm long. The inside of the dipper was white to facilitate detection of larvae.

Sampling methods: Three types of tires were evaluated: A) light truck, 8-ply non-radial, 70 cm diam × 17 cm wide; B) radial automobile, 56 × 17.5 cm, and C) tractor, 128 × 70 cm. When standing and filled with water, their capacities were 4, 3 and 28 liters, respectively.

For each tire, 20, 50, 100, 150 and 200 each of 2nd- to 4th-instar *Ae. aegypti* from the

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Queensland Institute of Medical Research colony were introduced separately into different water levels: full, ⅔ full and ⅓ full. The net was inserted sideways into the tire, twisted 90° and swept through the water as close to the inner sides as possible. This was achieved by pushing down on the net frame to expand it. Each sweep took approximately 5 sec with an interval of at least 5 min between sweeps. The number of replicates carried out for each trial was usually 10 but varied between 6 and 12, depending on the availability of larvae. Larvae were counted in a white tray and healthy ones reintroduced into the tire. Damaged larvae were replaced with fresh ones. This procedure was also carried out in situations with and without organic sediments in the base of the tires. Sediment comprised a 1:2 mixture of leaves and soil that filled approximately half of the volume. Leaves were not put back into the tires on successive samples so the sediment volume at the end of a sampling series was between ⅓ and ½ of that originally introduced. Water temperature for these trials was 26 ± 1°C.

For dipping, which took approximately 2 sec, the collector directed the ladle to where larvae could be seen as is usually done in field surveys. The number of dips, interval between dips and general methodology described for sweeping was carried out for dipping. Recovery rates under different situations were analyzed by stepwise multiple regression, ANOVA, correlation coefficient and student's t-test using SAS statistical software.

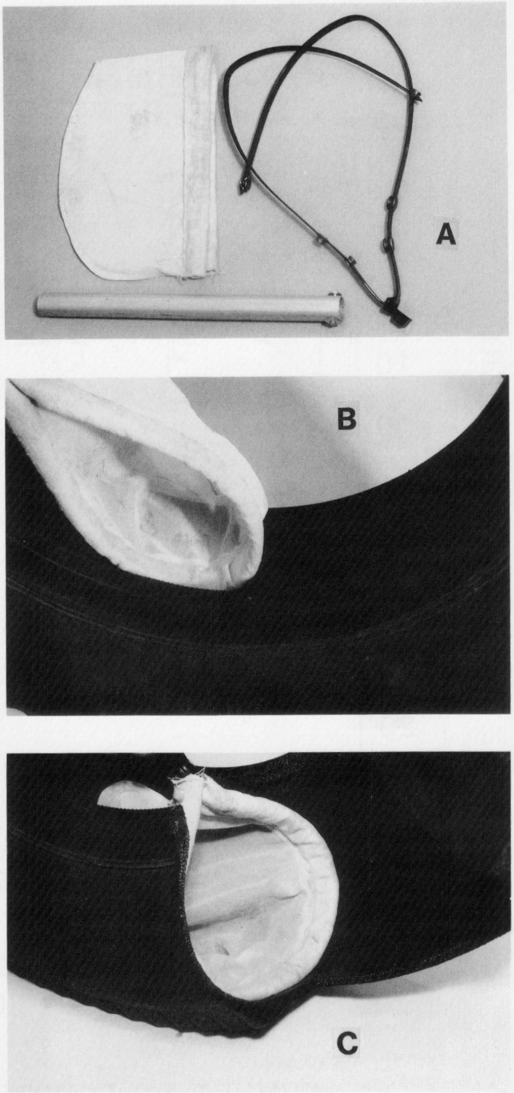

Fig. 1. The net and frame (A) and mode of use in the tire. The net is inserted sideways (B) and then rotated 90° in order to sample (C). Note the expansion of the frame when inside the tire.

RESULTS

Relative sensitivity and recovery rates by method: In all cases, 2–6.8 times more larvae were sampled by net than dipper ($t = 41.6, P = 0.0001$). In initial trials, mean percentage recoveries in the 3 tire sizes varied from 23.6 to 37.7% for sweeping and 5.2 to 11.0% for dipping. Sweeping gave more consistent results than dipping with the coefficients of variation ranging from 12.2 to 31.1 and from 38.1 to 62.4, respectively.

The correlation coefficient for $\log(x + 1)$ of numbers collected against number introduced for sweeping ($r = 0.9$, $n = 787$, $P < 0.0001$) was higher than that for dipping ($r = 0.79$, $n = 787$, $P < 0.0001$). The number collected by the 2 methods gave a positive correlation ($r = 0.85$, $n = 787$, $P < 0.0001$), as expected.

Regarding the sensitivity of the 2 methods, none of the tires with larvae were negative by net. Using a dipper, 20 of 158 trials (12.7%) with 20 larvae were negative and 3 of 166 (1.8%) were negative when 50 larvae were present.

Factors influencing recovery: From a step-wise multiple regression analysis of $\log(x + 1)$ transformed data from the sweeping trials, sediment (F = 417.3, df = 1, $P < 0.0001$), tire size (F = 112.2, df = 2, $P < 0.0001$), water level (F = 110.2, df = 2, $P < 0.0001$), larval number (F = 52.1, df = 4, $P < 0.0001$) and instar level (F = 6.9, df = 2, $P < 0.008$) significantly affected percent recovery. These 5 factors accounted for 53.4% of the variability in the model ($r^2 = 0.534$, $n = 787$, $P < 0.0001$).

For dipping, all factors except larval numbers (F = 2.8, df = 4, $P = < 0.09$) were significant as follows: tire size (F = 141.6, df = 2, $P < 0.0001$),

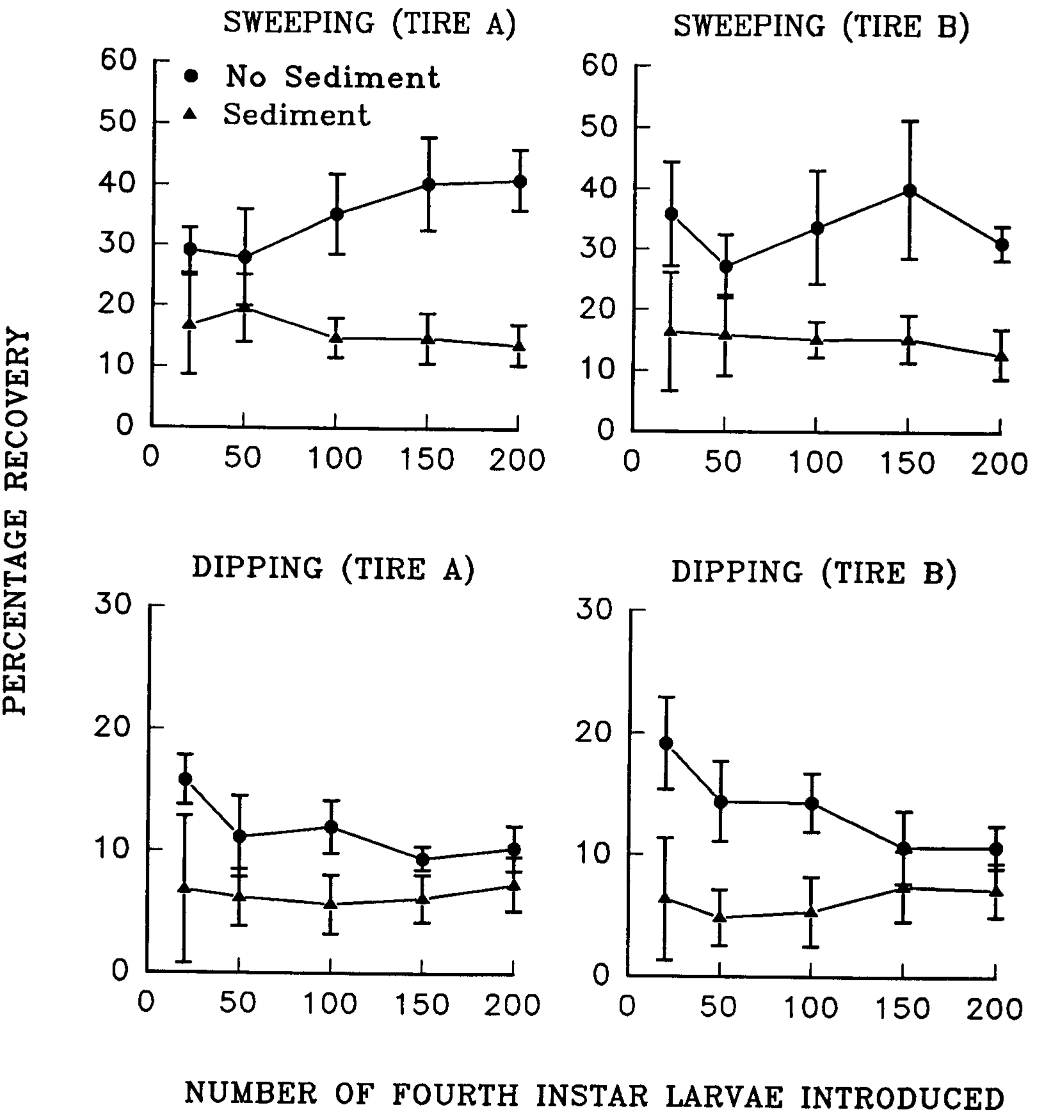

Fig. 2. Effect of sediment on percentage recovery of *Aedes aegypti* larvae by sweeping and dipping in tires A (light truck) and B (automobile).

water level ($F = 77.8$, df = 2, $P < 0.0001$), sediment ($F = 55.3$, df = 1, $P < 0.0001$), and instar level ($F = 14.5$, df = 2, $P < 0.0002$). Together these factors accounted for 29.6% of the variability in the model ($r^2 = 0.2956$, $n = 787$, $P < 0.0001$).

Sediment: For sweeping, in the presence of 4th-instar larvae, sediment in the tires was the most important factor influencing recovery rates but it was only 3rd in importance for dipping (Fig. 2). When sediment was present, catches by net were 2.3 times lower overall (e.g., in tire A without sediment, the average recovery was 37.7 ± 9.4% but this was reduced to 16.3 ± 5.6%). By dipper, average catch in tire A fell from 9.6 ± 4.1% to 6.4 ± 3.4%.

Tire size: For both sweeping and dipping, tire size was the 2nd most important factor. For the 3 tires with 3–28 liters capacity, the average difference in percentage recovery in the initial trials was greater for dipping (113% increase) than for sweeping (59%). These trends are consistent when different water levels (Table 1) and instars (Table 2) are also considered. For tires A and B, there was no significant difference between percentage recoveries by sweeping ($t = 1.56$, df = 543, $P >$

Table 1. Percentage recovery (±SD) of different instars of *Ae. aegypti* larvae in different water levels in tires without sediment.

Method	Tire	No. samples	Water level Full	⅔ full	⅓ full
Sweeping	A	272	31.9 ± 12.8	45.8 ± 11.1	43.1 ± 11.7
	B	272	29.0 ± 12.2	47.0 ± 10.8	40.2 ± 10.5
	C	240	23.6 ± 7.3		36.4 ± 11.5
Dipping	A	272	8.7 ± 4.1	14.1 ± 4.0	18.7 ± 9.4
	B	272	9.6 ± 4.5	16.3 ± 5.3	20.8 ± 10.2
	C	240	5.2 ± 3.2		8.9 ± 4.1

0.05) but more larvae were always sampled from tire B than A when dipping (t = 2.55, df = 543, P < 0.05).

Water level: The volume of water inside the tire was the most important factor affecting recovery rates using a dipper but was the 3rd most important factor affecting sweeping. For sweeping and dipping, respectively, recoveries were highest when the tires were ⅔ and ⅓ full (Table 1), but both of these gave higher percentage returns compared to full tires.

Number of larvae introduced: In relation to the models developed for both sweeping (P < 0.001) and dipping (P = 0.09), this factor had a minor influence compared to the 3 presented above (Fig. 2).

Larval instar: Although differences in recovery rates of 2nd-, 3rd- and 4th-instar larvae were significant for both methods, this factor was of minor importance to the regression models. The percentage recovery of 2nd-instar larvae (Table 2) was lower than for the other instars tested by either sweeping (F = 85.3, df = 2, P < 0.001) or dipping (F = 45.6, df = 2, P < 0.001).

DISCUSSION

The net described herein is light, easily carried and inexpensive (U.S. $3) and if necessary, can be fitted with an aluminum handle for more general sampling purposes. For sampling water inside tires, the cable ties extending above the netting could be comfortably used for this purpose. The pigskin leather around the flexible frame ensures its durability.

In surveying container habitats, the main objective is usually detection of immature stages of *Ae. aegypti* and other species, in order to define risk using a Breteau index (Breteau 1954). With respect to the 3 tire sizes chosen under a variety of conditions, we demonstrated that this net would detect immatures more consistently than a dipper. In our hands, all net samples were positive, whereas dipping returned 20 negative from 158 (12.7%) trials with 20 larvae introduced. With 50 larvae introduced, 3 of 166 (1.8%) samples were negative by dipping. Thorough detection of breeding sites is a prerequisite of successful and lasting control, especially in the latter stages of a campaign when larval densities are low.

For this reason, we chose to introduce and evaluate sampling efficacy with respect to 20–200 larvae. It is also relevant that in north Queensland (Tun-Lin 1992[3]) and in Fiji (Andre et al. 1992[4]), the average number of *Ae. aegypti* larvae per tire was 90 (n = 68) and 157 (n = 68), respectively, and in Louisiana, Marten (1990) recorded an average of 142 *Ae. albopictus* per tire. Hence, we believe that the range chosen for our evaluation applies to natural conditions but acknowledge that tires with fewer or greater numbers of immatures do exist.

With respect to estimation of productivity, the lower coefficients of variation of sweeping, usually less than the mean recovery rates, indicated that it is more reliable than dipping, where coefficients of variation ranged from 3.4 to 11.9 times higher. In view of the importance of the presence of sediment in the bottom of the tire, tire size, and water volumes, we were surprised that there was such a high correlation (r = 0.89, n = 787, P < 0.001) between numbers sampled and numbers introduced. However, we have not included the regression formulae because of the relatively small number of variables examined.

Because the net could sample almost all of the water in the radial automobile and the 8-ply light truck tires, its efficiency was clearly higher than in the larger tractor tire, which was more than 4

[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. thesis. Tropical Health Program, University of Queensland.

[4] Andre, R. G., B. H. Kay, E. Kikau and S. H. Waterman. 1992. Assessment of the epidemiology of dengue and its past, present and future control in Fiji. Vector Biology and Control Project Report No. 81269, Arlington, VA.

Table 2. Percentage recovery (±SD) of different instars of *Ae. aegypti* in tires full of water and without sediment, by sweeping and dipping.

Method	Tire	No. samples	Larval instar II	III	IV
Sweeping	A	131	37.1 ± 9.1	40.1 ± 10.6	36.0 ± 8.1
	B	131	33.8 ± 10.1	35.0 ± 9.7	34.1 ± 8.8
	C	120	18.2 ± 5.2	27.2 ± 6.3	25.6 ± 7.1
Dipping	A	131	8.0 ± 3.9	10.3 ± 4.5	11.4 ± 2.9
	B	131	9.7 ± 3.9	10.9 ± 4.0	13.1 ± 4.1
	C	120	4.4 ± 3.0	6.7 ± 3.5	4.4 ± 2.6

times wider. Overall the net collected 4 times more than the dipper. However, sweeping was more affected by the presence of bottom sediments because, in contrast to dipping, which primarily took a surface sample, the net sampled the total water column. Recovery rates were higher with both methods when water levels were reduced but it appears likely that the sweeping procedure is hindered somewhat with small volumes. The edges of a dipper are finer than those of a net and are more able to scoop up anything remaining. Although more immatures were always collected by dipper in tire B (nonradial truck tire) than in tire A (radial automobile tire), shape was also important to recovery, but for sweeping this was unimportant because the net was able to accommodate the different shapes.

More 3rd- and 4th-instar larvae were collected than 2nd instars, as was found by Tun-Lin (1992[3]) in 220-liter drums. It is well established that first and second instar *Ae. aegypti* will remain submerged for longer periods than any of the other stages, particularly at cooler temperatures (Christophers 1960, Tun-Lin 1992[3]).

We recommend the use of the flexible net for surveys of container-breeding *Aedes* as a means of reducing effort and increasing precision, especially as we have frequently encountered difficulty in inserting dippers and removing samples where the gap between the tire walls is narrow. As most personnel will only be interested in establishing presence or absence of immatures, this net saves time. It also has the dual benefit of being suitable for sampling predacious copepods, promising biological control agents for *Aedes* (Rivière et al. 1987, Marten 1990).

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