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The single water-surface sweep estimation method accurately estimates very low (n = 4) to low–moderate (n = 25–100) and high (n > 100) *Aedes aegypti* (Diptera: Culicidae) pupae numbers in large water containers up to 13 times faster than the exhaustive sweep and total count method and without any sediment contamination

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Abstract

OBJECTIVE To confirm that a single water-surface sweep-net collection coupled with three calibration factors (2.6, 3.0 and 3.5 for 1/3, 2/3 and 3/3 water levels, respectively) (WSCF) could accurately estimate very low to high *Aedes aegypti* pupae numbers in water containers more rapidly than the exhaustive 5-sweep and total count (ESTC) method recommended by WHO.

METHODS Both methods were compared in semi-field trials using low (n = 25) to moderate (n = 50–100) pupae numbers in a 250-l drum at 1/3, 2/3 and 3/3 water levels, and by their mean-time determinations using 200 pupae in three 220- to 1024-l water containers at these water levels. Accuracy was further assessed using 69.1% (393/569) of the field-based drums and tanks which contained <100 pupae.

RESULTS The WSCF method accurately estimated total populations in the semi-field trials up to 13.0 times faster than the ESTC method (all P < 0.001); no significant differences (all P-values ≥ 0.05) were obtained between the methods for very low (n = 4) to low–moderate (n = 25–100) and high (n > 100) pupae numbers/container and without sediment disturbance.

CONCLUSION The simple WSCF method sensitively, accurately and robustly estimated total pupae numbers in their principal breeding sites worldwide, containers with >20 l water volumes, significantly (2.7- to 13.0-fold: all P-values <0.001) faster than the ESTC method for very low to high pupae numbers/container without contaminating the clean water by sediment disturbance which is generated using the WHO-recommended ESTC method. The WSCF method seems ideal for global community-based surveillance and control programmes.

keywords *Aedes aegypti*, pupae, estimation, water-surface sweeping, calibration factor, total counts

Introduction

The development of a method to rapidly and accurately determine *Aedes aegypti* pupae (and larvae) populations in their principal breeding sites, large water containers, has been a major priority for dengue virus surveillance and control. Two methods were initially assessed for this purpose (Tun-Lin *et al.* 1994; Kubota *et al.* 2003). In these semi-field trials, dipping and sweep-net coupled with calibration factors were derived for *Ae. aegypti* larvae and pupae estimations in 220-l metal drums at three different water levels (Tun-Lin *et al.* 1994), and an optimally derived 8-sweep-net method performed throughout the water within 80-l drums was used to collect 72% of the *Ae. aegypti* larvae (Kubota *et al.* 2003). For these trials, each test was repeated 10 times to obtain accurate results. This sweep-net method was used to derive new calibration factors using only three repeats per test, but the results were not provided (Villegas *et al.* 2006) nor subsequently published. Later, a 5-sweep-net method was performed throughout the water in seven water container types at three different water levels to derive calibration factors for *Ae. aegypti* larvae and pupae estimations (5-SCF method) in semi-field conditions (Knox *et al.* 2007). The pupae were then collected and counted using a multiple 'exhaustive' 5-sweep-net and total count (ESTC) method repeated only five times in semi-field conditions and in 343 of 406 *Ae. aegypti* pupae-positive containers compared with total counts for all (n = 406) of them in field trials (Knox *et al.* 2007). Only low or very low linear regression correlations were, however, obtained for larvae (R^2 = 0.443–0.610) and pupae (R^2 = 0.282–0.328) estimations, respectively (Knox *et al.* 2007).

By contrast, a single water-surface sweep-net procedure coupled to three calibration factors (WSCF) method for 1/3, 2/3 and 3/3 (full) water levels accurately estimated *Ae. aegypti* pupae numbers in large (>20 l water volume) containers of different sizes and shapes even when their mean pupae numbers were low and ranged from (i) 26 (95% CI: 20–34) for 220- to 400-l drums of which 91% contained water and 55% were full, (ii) to 37 (95% CI: 31–44) for 40- to 4550-l ground tanks of which 95% contained water and 61% had a 2/3 water level and (iii) 40 (95% CI: 13–122) for 360- to 6060-l elevated tanks of which 93% contained water and 88% were full (Romero-Vivas *et al.* 2002). Despite these findings, some doubt remained that a universal rapid pupae sampling method could be derived because such calibration factors would need to be differentially derived for different water container types, and derivation in one location could give faulty estimates in another location and would differ due to 'subtle climatic differences between different locations' (Focks & Alexander 2006). Thus, this WSCF method was further evaluated in four locations (14–1630 m above sea level) covering the *Ae. aegypti* breeding temperature (19–28 °C) range using: (i) locally obtained *Ae. aegypti* pupae and (ii) the most productive *Ae. aegypti* breeding container types (large (>1000 l) and moderately large (≤1000 l) ground tanks and 220-l drums) at three different (1/3, 2/3 and 3/3) water levels (Romero-Vivas *et al.* 2007). In this study, only three calibration factors were again required for each water level, irrespective of the actual water volumes in these differently sized containers, throughout the *Ae. aegypti* breeding temperature range, and a high correlation (R^2 = 0.72) was obtained between the estimated and total pupae numbers (Romero-Vivas *et al.* 2007). This simple, rapid, accurate, sensitive and robust WSCF method was therefore adopted by the Ministry of Health for use in *Ae. aegypti* surveillance and control throughout Colombia (Ministerio de la Proteccción Social 2011).

WHO subsequently published 'The Operational guide for assessing the productivity of *Ae. aegypti* breeding sites' (Manrique-Saide *et al.* 2011) which suggests that the WSCF method should only be used for large (>20 l) water containers that contained >100 *Ae. aegypti* pupae. By contrast, the WHO (Manrique-Saide *et al.* 2011) suggested that for the large containers with >20 l water volumes and <100 *Ae. aegypti* pupae that could not be emptied for total pupae counts, they should be collected using a 20-cm-diameter × 30-cm-deep net by the 'exhaustive' 5-sweep-net technique coupled with the total count (ESTC) method (Knox *et al.* 2007). In the WHO guidelines (Manrique-Saide *et al.* 2011), however, this method was termed 'comprehensive netting' without adequately providing credit to those who designed the net and fully assessed their ESTC method (Knox *et al.* 2007), nor to those who designed another sized net, derived the three calibration factors and fully assessed their WSCF method (Romero-Vivas *et al.* 2007). Of particular note was that: (i) total *Ae. aegypti* pupae counts performed by emptying these containers or using the ESTC method, as suggested by WHO (Manrique-Saide *et al.* 2011), would take considerably longer than the WSCF method and (ii) both of these total count methods would contaminate clean water through sediment (detritus) layer disruption and thus be unacceptable to owners (Kubota *et al.* 2003).

We therefore further evaluated our WSCF method to accurately estimate low *Ae. aegypti* pupae numbers in large water containers in semi-field (n = 25–100 and 200) and field (n < 100) trials and determined and compared the mean times taken to perform these WSCF and ESTC methods.

Materials and methods

Sampling methods

Briefly, using the water-surface sweep-net and calibration factor (WSCF) method for *Ae. aegypti* pupae estimations (Romero-Vivas *et al.* 2007), we performed one gentle sweep, using a 15-cm-diameter × 20-cm-deep net immersed half-way (7.5 cm) beneath the water surface, on any type of large (>20 l water volume) container. The *Ae. aegypti* pupae numbers collected were then multiplied by either 2.6, 3.0 or 3.5 for containers that had 1/3, 2/3 or 3/3 water levels, respectively, to obtain their total estimated numbers.

The total *Ae. aegypti* pupae present in different types of large water containers in three neighbourhoods of Barranquilla (Colombia) were collected using a 40 × 40 cm and 50 cm deep net with a mesh size of 1 × 1 mm and counted after being transferred to a white plastic bowl as described (Romero-Vivas *et al.* 2006).

Small (n = 25) and moderate (n = 50, 75 and 100) locally reared *Ae. aegypti* pupae numbers were placed in a 250-l round plastic drum (barrel) at different (1/3, 2/3 and 3/3) water levels. The WSCF method was then performed 10 times for each trial to adequately assess its accuracy and any damaged pupae were replaced.

The information collected on 569 different types of large water containers (cement ground tanks and cement, metal and plastic drums) surveyed for pupae productivity in three neighbourhoods of Barranquilla using the WSCF and the total count methods was compared using only those containers that contained <100 pupae (Romero-Vivas *et al.* 2007).

The derivation and full evaluation of the WSCF method has been described before (Romero-Vivas *et al.* 2007). The time taken to estimate and count the total pupae numbers using the WSCF and ESTC methods, using the same 15 cm (diameter) × 20 cm (deep) net, was measured at three (1/3, 2/3 and 3/3) water levels in three different water containers: (i) a 220-l capacity metal drum (surface area 0.38 m^2), (ii) a cement ground tank (GT1: ≤1000 l capacity) with a 520 l capacity (surface area: 0.48 m^2) and (iii) a cement ground tank (GT2: >1000 l capacity) with a 1024 l capacity (surface area: 0.58 m^2). In these semi-field studies, 200 pupae were introduced into each container type, each test was repeated 10 times to adequately compare these two methods, and any damaged pupae were replaced and each repeat was performed after 5 min.

Statistical analysis

The Student's *t*-test was used to compare the 95% confidence intervals and assess levels of significance (*P*-values) between the WSCF and total count methods. Differences between the Student's *t*-test and the *z*-test results were also assessed with the data. The Spearman rho *P*-values were also determined to further ensure accuracy and robustness of the WSCF and total count methods in containers with very low to high pupae numbers.

Results

In this study, the overall mean percentages estimated compared to the total pupae numbers were 94.1% (σn = 5.0), with mean values of 90.1% (σn = 7.1), 88.2% (σn = 12.8) and 98.0% (σn = 17.1) for 1/3, 2/3 and 3/3 water levels, respectively (Table 1). The overall mean WSCF method percentages estimated using 25, 50, 75 or 100 pupae were 97.8% (σn = 16.5), 86.6% (σn = 3.4), 101.4% (σn = 13.5) and 91.8% (σn = 20.8), respectively. Importantly, with the exception of a slight overlap between the results for 25 and 50 pupae at 3/3 water levels (95% CI: 24.6–32.8 and 32.4–49.6) and 50 and 75 pupae at 1/3 water levels (95% CI: 32.3–55.0 and 51.1–72.6), there was no overlap between the 95% CI values performed at different water levels with 25 (low), 50 or 75 (moderate) *Ae. aegypti* pupae. Thus, this WSCF method was very accurate and robust for low to moderate pupae number estimations. (see *z*-test and Spearman rho *P*-values below).

Of the 569 large water containers assessed in field studies (Romero-Vivas *et al.* 2007), the majority (69.1%: 393/569) contained <100 *Ae. aegypti* pupae (Table 2). Four types of large water containers were classified as follows: (i) cement ground tanks (n = 124), (ii) cement drums (n = 52), (iii) metal drums (n = 61) and (iv) plastic drums (n = 156). The lowest water volume ranges were identified in metal drums (69–340 l), followed by plastic

Table 1 Assessment of the WSCF method to accurately estimate from low (n = 25) to moderate (50–100) *Aedes aegypti* pupae numbers in a drum at different water levels in semi-field conditions

	Mean number (95% CI) and % of 25, 50, 75 or 100 *Ae. aegypti* pupae estimated from using the WSCF method in a 250-l drum							
	25		50		75		100	
Water level	Mean numbers (95% CI)	Mean %	Mean numbers (95% CI)	Mean %	Mean numbers (95% CI)	Mean %	Mean numbers (95% CI)	Mean %
1/3	23.9 (17.9–29.9)	95.6	43.7 (32.3–55.0)	87.4	61.9 (51.1–72.6)	82.5	78.8 (65.1–92.4)	78.8
2/3	19.8 (14.8–24.2)	78.7	45.1 (29.3–60.7)	90.2	81.1 (69.2–92.8)	108.3	75.5 (65.0–85.0)	75.5
3/3	28.7 (24.6–32.8)	114.8	41.2 (32.4–49.6)	82.1	85.2 (65.4–104.1)	113.3	121.1 (104.8–137.4)	121.1
Total mean	24.1 (20.9–27.2)	96.4	43.3 (33.1–53.1)	86.6	76.1 (66.8–85.2)	101.4	91.8 (80.9–102.4)	91.8

The sweep net dimensions were 15 cm diameter × 20 cm deep, and the calibration factors for 1/3, 2/3 and 3/3 water levels were 2.6, 3.0 and 3.5 as described previously (Romero-Vivas *et al.* 2007); each test was repeated 10 times, and the Student's *t*-test was applied to the resultant data.

Table 2 Water level (1/3, 2/3 and 3/3) and volume (l) ranges and means in cement ground tanks and cement, metal and plastic drums identified with less than 100 *Aedes aegypti* pupae in the field studies

	Cement ground tanks			Cement drums			Metal drums			Plastic drums		
Water level	*n*	Water volume	Mean (95% CI)	*n*	Water volume	Mean (95% CI)	*n*	Water volume	Mean (95% CI)	*n*	Water volume	Mean (95% CI)
1/3	28	160–2610	1082 (811–1353)	15	67–510	218 (154–282)	13	75–307	228 (192–264)	32	25–700	177 (138–217)
2/3	52	154–6412	1381 (1066–1697)	18	120–829	313 (210–415)	21	120–340	239 (215–262)	59	26–254	150 (134–166)
3/3	44	168–2289	1080 (770–1389)	21	104–477	351 (161–542)	27	69–254	237 (218–256)	65	20–632	146 (123–169)
Total/ Mean	124	160–6412	1207 (1046–1368)	52	67–829	305 (230–380)	61	69–340	236 (224–248)	156	20–700	154 (140–168)

The Student's *t*-test was applied to all of the data.

drums (20–700 l), cement drums (67–829 l) and cement ground tanks (160–6412 l).

The mean *Ae. aegypti* pupae numbers estimated using the WSCF method and collected and counted (total count) in all water container types at different water levels that contained <100 pupae in the field studies (Table 2) were compared (Student's *t*-test *P*-values) (Table 3). In this study, the lowest and highest numbers of *Ae. aegypti* pupae present within the 95% CIs were 3.8 (cement drums at 2/3 water level) and 40.2 (metal drums at 2/3 water level). As such, this evaluation was performed using very low ($n = 4$) to low–moderate ($n = 40$) *Ae. aegypti* pupae numbers naturally present in these large field-based water containers. Interestingly, despite the large size of the cement ground tanks which contained 160–6412 (mean = 1207) litres of water (Table 2), the mean WSCF method estimations/total counts for pupae were very similar at 1/3 ($n = 17.6/20.2$), 2/3 ($n = 15.2/20.3$) and 3/3 ($n = 16.6/18.0$) water levels (Table 3). Likewise, for the plastic drums, which had the lowest mean (154 l) water volumes (Table 2), the mean WSCF method estimations/total pupae counts were also similar for 1/3 ($n = 11.6/13.4$), 2/3 ($n = 11.8/8.6$) and 3/3 ($n = 17.1/13.3$) water levels (Table 3). Similar values were also obtained for the other two container types (cement drums and metal drums). Student's *t*-test values ranged from 1.8 to 1.9, but there was no significant difference (all *P*-values > 0.05) between the estimated and the total counted pupae numbers for any of these four container types at any water level when they contained very low ($n = 4$) to low–moderate ($n = 40$) *Ae. aegypti* pupae numbers. All *P*-values for these data using the *z*-test were also >0.05 (data not shown).

Importantly, the WSCF method did not disturb the sediment (detritus) layer and thus did not contaminate the upper clean water in water containers, as was observed in many field-based containers when pupae were collected using the extensive sweeping method.

In a cross-check to ensure that that the ratio of the sweep-net-collected pupae to the total pupae numbers in each container was not dependent upon on the total pupae numbers present, we found no significant differences (Spearman rho *P*-value > 0.05 in all cases) between these two methods regarding very low ($n = 4$), low ($n = 25$), moderate ($n = 50$–100) or high (>100) pupae numbers in these studies and those described previously (Romero-Vivas *et al.* 2007).

The mean time taken to perform the WSCF method ranged from 1.1 (>1000 l cement ground tank at 3/3 water level) to 4.1 (<1000 l cement ground tank at 1/3 water level) minutes (Table 4). By contrast, the mean time taken to perform the ESTC method ranged from 7.6

Table 3 Comparison of the means using *t*-test and *P*-values of *Aedes aegypti* pupae numbers estimated using the WSCF method and total counted in different container types with different water levels and less than 100 pupae in the field studies

		Mean (95% CI), *t*-test and *P* values for *Ae. aegypti* pupae estimated by the water-surface sweeping method coupled with calibration factors and the total counted for each container type in field studies											
		Cement ground tanks			Cement drums			Metal drums			Plastic drums		
Water level	Enumeration method	*n*	Mean (95% CI)	*t*-test (*P*)	*n*	Mean (95% CI)	*t*-test (*P*)	*n*	Mean (95% CI)	*t*-test (*P*)	*n*	Mean (95% CI)	*t*-test (*P*)
1/3	Sweeping	28	17.6 (7.3–27.8)	1.0 (0.33)	15	11.6 (4.6–18.6)	1.5 (0.16)	13	10.6 (1.9–19.3)	0.4 (0.70)	32	11.6 (4.4–18.8)	0.8 (0.42)
	Total counting		20.2 (9.9–30.4)			16.2 (4.4–28.0)			11.9			13.4	
2/3	Sweeping	52	15.2 (8.1–22.3)	1.9 (0.06)	18	17.8 (3.2–32.5)	−0.8 (0.44)	21	27.3 (7.1–47.4	−0.6 (0.56)	59	11.8 (6.5–17.2)	−1.9 (0.06)
	Total counting		20.3 (13.7–26.9)			14.7 (3.8–25.6)			25 (9.8–40.2)			8.6 (5.9–11.4)	
3/3	Sweeping	44	16.6 (9.3–23.9)	1.0 (0.31)	21	16.3 (5.5–27.0)	−1.7 (0.10)	27	9.3 (3.9–14.7)	1.1 (0.28)	65	17.1 (10.7–23.5)	−1.8 (0.07)
	Total counting		18 (11.9–24.0)			11.6 (5.8–17.4)			12.4 (4.5–20.3)			13.3 (9.2–17.5)	
Total/ Mean	Sweeping	124	16.8 (12.5–21.1)	0.3 (0.76)	52	15.7 (9.4–21.3)	−0.9 (0.36)	61	16.6 (9.8–23.4)	0.8 (0.42)	156	14 (10.3–17.7)	−0.7 (0.48)
	Total counting		18.5 (14.8–22.3)			13.8 (9.2–18.5)			18 (12.3–23.7)			11.6 (9.1–14.0)	

The sweep net dimensions were 15 cm diameter and 20 cm deep and the calibration factors used for 1/3, 2/3 and 3/3 water levels were 2.6, 3.0 and 3.5 respectively, while the *Ae. aegypti* pupae for the total count were collected using full container sweeping using a large net of 40 cm × 40 cm (square) × 40 cm deep as described previously (Romero-Vivas *et al.* 2007). The Student's *t*-test was applied to all of the resultant data.

(metal drum at 2/3 water level) to 19.9 (>1000 l cement ground tank at 2/3 water level) minutes. The mean times for performing the WSCF *vs.* the ESTC methods showed the WSCF to be 3.3, 4.0 and 6.6 times faster to perform (all *P*-values <0.001) for the metal drum, the 520- and the 1024-l cement ground tanks at each water level. These time differences were greatest for the large cement ground tanks (13.0 fold faster) at 3/3 water volumes due to the ease of performing the WSCF method when these very large tanks were full, while being the slowest to perform the ESTC method.

Discussion

The dengue virus vector species, *Ae. aegypti,* is predominantly a domestic mosquito whose principal breeding sites are water storage containers. Our WSCF method was accurate and robust and did not disturb the sediment (detritus) layer found at the bottom of many of these containers as occurs when using the 8-sweep method (Kubota *et al.* 2003), the 5-sweep with calibration factor (5-SCF) method (Knox *et al.* 2007), the ESTC method (Knox *et al.* 2007) or the total count method after emptying the containers as was suggested by WHO (Manrique-Saide *et al.* 2011) (see below).

The WSCF method (Romero-Vivas *et al.* 2006, 2007) and 5-sweep procedure coupled with calibration factors (5-SCF) method (Knox *et al.* 2007) were further assessed in semi-field conditions in São Paulo, Brazil, using 200, 500 and 1000 l capacity containers (Regina Dibo *et al.* 2013). In that study, the WSCF method however required very different calibration factors for *Ae. aegypti* pupae when they were performed using different pupae numbers compared to different larvae:pupae ratios of 9:1. Different calibration factors were also derived when the single water-surface sweep was performed for the drum and the two water tanks at different water levels (Regina Dibo *et al.* 2013). The low number ($n = 5$) of repeats/test, as was used previously (Knox *et al.* 2007), may have contributed to these findings. By contrast, our WSCF method was very accurate and robust in semi-field and field studies reported in this study and previously (Romero-Vivas *et al.* 2006, 2007). We clearly showed that only three calibration factors were required to accurately and robustly estimate the *Ae. aegypti* pupae numbers in all shapes and capacities of semi-field and field-based water containers with either 1/3, 2/3 or 3/3 water levels, even when they were used at different locations with different ambient temperatures using locally collected *Ae. aegypti* pupae (Romero-Vivas *et al.* 2007). Our WSCF method was also robust between the 10 teams and the individuals within each team who

Table 4 Mean and time (fold) differences to enumerate *Ae. aegypti* pupae in ground tanks and a drum using the WSCF and ESTC methods in semi-field trials

Container type (Capacity)	Water level	Mean pupae numbers collected in one sweep	Mean time (95% CI) in minutes for pupae enumerations by		Mean time difference for total/estimated enumerations (fold)
			WSCF method	ESTC method	
Metal drum (220 l)	1/3	93.2	2.7 (2.4–3.1)	8.5 (8.0–8.9)	3.1
	2/3	83.6	2.6 (2.2–3.1)	7.6 (6.9–8.2)	2.9
	3/3	70.6	2.5 (2.2–3.1)	10.0 (8.6–11.4)	4.0
Subtotal		82.5	2.6 (2.4–2.8)	8.7 (8.1–9.3)	3.3
Cement ground tank (≤1000 l)	1/3	102.3	4.1 (3.4–4.7)	11.0 (8.6–13.5)	2.7
	2/3	89.4	3.7 (3.3–4.1)	13.5 (12.0–14.9)	3.6
	3/3	57.1	1.8 (1.6–2.0)	13.7 (12.1–15.2)	7.6
Subtotal		82.9	3.2 (2.8–3.6)	12.7 (11.6–13.9)	4.0
Cement ground tank (>1000 l)	1/3	89.4	3.1 (2.2–4.0)	13.5 (12.6–14.5)	4.4
	2/3	59.8	3.0 (2.5–3.5)	19.9 (17.7–22.2)	6.6
	3/3	52.6	1.1 (1.7–2.2)	14.3 (13.2–15.5)	13.0
Subtotal		66.9	2.4 (1.9–2.9)	15.9 (14.6–17.3)	6.6

200 *Ae. aegypti* pupae were used in each the container type, the sweep net dimensions were 15 cm diameter × 20 cm deep, and the calibration factors of 2.6, 3.0 and 3.5 were used for water levels of 1/3, 2/3 and 3/3, respectively (Romero-Vivas *et al.* 2007), while the same net was used for the ESTC method reported previously (Knox *et al.* 2007); each test was repeated 10 times, and the Student's *t*-test was applied to the resultant data.

applied this method in field studies (Romero-Vivas *et al.* 2007).

WHO has suggested that the ESTC method should be performed using a small (20-cm-diameter × 30-cm-deep) net in containers with >20 l of water 'if emptying was not feasible (due to the size or nature of the container)' when they were believed to contain <100 pupae (Manrique-Saide *et al.* 2011). In our study, the ESTC method required significantly more time to perform (Table 4), thereby adding unnecessary additional costs to *Ae. aegypti* pupae/larvae control programmes and generating owner-unacceptable sediment (detritus) layer disturbance/disruption as reported previously (Kubota *et al.* 2003). In one Colombian study, many owners opted to destroy their large water tanks to prevent the inspectors: (i) unnecessarily spending additional time emptying them for total pupae counts and (ii) contaminating the upper clean water in them (CM Romero-Vivas and AK Falconar, unpublished observation).

Despite the WSCF method previously being shown to accurately and robustly estimate low (<100) *Ae. aegypti* pupae numbers in all water containers with >20 l of water (Romero-Vivas *et al.* 2007), in a recent WHO-funded study: (i) the ESTC method was performed in all containers with 20–100 l water volumes and (ii) the WSCF method was only used for large containers with >100 l water volumes (Quintero *et al.* 2014). Thus, these teams opted to use these methods despite barrels (drums) and tanks being important *Ae. aegypti* breeding sites in each of these study sites located in five Latin American countries (Quintero *et al.* 2014), thereby resulting in unnecessary assessment times for 20- to 100-l water containers and owner-unacceptable sediment (detritus) layer disruption/disturbance (Kubota *et al.* 2003). Such 'top-down' suggestions by WHO (Manrique-Saide *et al.* 2011) were, therefore, criticised due to being time-consuming and expensive, as well as being ineffective due to the exclusion of community involvement ('bottom-up' approach) (Gubler 1989, 2002, 2005). WHO-suggested methods for *Ae. aegypti* pupae surveillance were published (Manrique-Saide *et al.* 2011) despite contrary expert advice (Gubler 1989, 2002 ,2005) and in disregard of numerous reports where local residents distrusted 'top-down' *Ae. aegypti* surveillance and control programme inspectors (Parks & Lloyd 2004; Phuanukoonnon *et al.* 2005; Espino *et al.* 2012; Tsuzuki *et al.* 2013). In our previous studies, we recruited many local residents within our 10 teams who were readily trained to use our WSCF method to rapidly, sensitively, accurately and robustly estimate both *Ae. aegypti* pupae and L3/4 larvae in their principal breeding sites (Romero-Vivas *et al.* 2007; Romero *et al.* 2010). There are now multiple reports where community education and participation in *Ae. aegypti* surveillance and control programmes were effective and sustainable (Abeyewickreme *et al.* 2012; Arunachalam *et al.* 2012; Tana *et al.* 2012; Wai *et al.* 2012). As large (>20 l) water containers are the principal *Ae. aegypti* breeding sites throughout Colombia, elsewhere in Latin

America (Nathan *et al.* 2006; Focks & Alexander 2006; Quintero *et al.* 2014) and worldwide (Rodhain 1996; Focks & Alexander 2006; Nathan *et al.* 2006), we believe this simple method is ideal for community-based *Ae. aegypti* surveillance and control programmes.

As there are multiple other errors, including the alteration of one of our calibration factors without validation in the WHO guidelines (Manrique-Saide *et al.* 2011) together with multiple un-validated theoretically derived methods which will be reported elsewhere, *Ae. aegypti* surveillance and control teams should be extremely cautious about employing the WHO-published pupae surveillance methods without thorough validations and appropriate comparisons.

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