Quantitative Relationships Between Immature and Emergent Adult *Aedes aegypti* (Diptera: Culicidae) Populations in Water Storage Container Habitats

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ABSTRACT Although quantitative surveillance data for immature stages of *Aedes aegypti* are often used to prioritize containers or specific types of containers for control, the relationship between immature and emergent adult populations under field conditions is largely unknown. We examined the relationships between abundance of III/IV instars and pupae, and emerging adult population for a series of water storage containers in southern Vietnam. A large proportion of III/IV instars failed to progress to adulthood, and the relationships between III/IV instars and adults were poor. Collected IV instars appeared to be nutritionally deprived, although their size and nutrient levels were not reliable indicators of emergence success. Conversely, pupal abundance was a good indicator of emerging adult populations, especially over the ensuing 48-h period. Although there were clear advantages of pupal surveillance over surveillance of III/IV instars for the estimation of adult mosquito productivity, there were practical limitations associated with the enumeration of pupae, and their comparatively low densities may preclude the identification of potentially productive containers.

KEY WORDS *Aedes aegypti*, larvae, pupae, emergence, productivity

In the absence of cost-effective methodologies for direct assessment of adult *Aedes aegypti* (L.) (Diptera: Culicidae) abundance, there has been a focus on measuring aquatic immature stages and emerging adult populations. Whereas the latter is important for ascribing epidemiological risk, the former is also important for prioritizing control of immature stages in particular container types (Focks and Alexander 2006), and measuring the efficacy of interventions. Also, given the likely development of new population replacement strategies that involve the release of modified mosquitoes (Phuc et al. 2007, Jeffery et al. 2009), there is an increasing need to refine field population estimates for *Ae. aegypti*. As direct estimates of adult *Ae. aegypti* population sizes are difficult to obtain, several studies have resorted to extrapolation of adult numbers based on standing crops of pupae (Focks and Chadee 1997, Strickman and Kittayapong 2003, Jeffery et al. 2009). The relationships between immature and adult abundance will determine the accuracy of these population estimates. As such, an understanding of the ecological dynamics of immature *Ae. aegypti* populations under field conditions is required to aid the interpretation and utilization of quantitative immature surveillance data (Tun-Lin et al. 1994, 1995, 2000; Knox et al. 2007).

Despite a surfeit of laboratory studies on the developmental biology of *Ae. aegypti*, there has been little investigation into the ecological dynamics of aquatic stages under field conditions (Service 1992). Southwood et al. (1972) presented one of the few life table analyses for *Ae. aegypti* with their longitudinal study of egg, larval, and pupal populations in water containers at a temple in Thailand. They found that the number of *Ae. aegypti* adults emerging from water jars, ant traps, and flower pot plates was influenced by the different mortalities within specific stadia. For water jars, the majority of mortality from hatching to adult emergence occurred between the IV instar and pupal stadia (October to February), or between eggs and II instars as a result of density-dependent effects (March to September). They also noted that many IV instars failed to develop to pupae, instead remaining at the IV instar for prolonged periods of time. Such stagnation at the IV instar would result in a profusion of IV instars with respect to pupae, as observed earlier for field populations in large water storage containers in Vietnam (Knox et al. 2007). This, along with significant mortality between the late immature stages and emergence, would limit predictions of emerging adult populations based solely on immature abundance esti-

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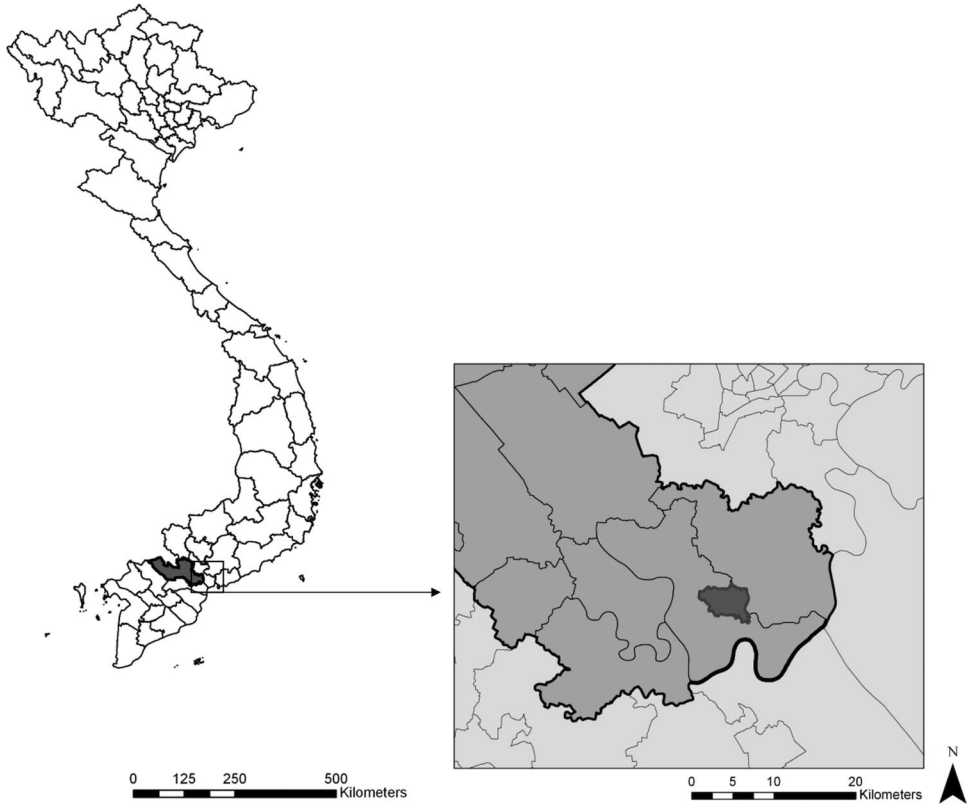

Fig. 1. Tan Lan commune (dark shading in right figure) in Can Duoc District, Long An province in southern Vietnam. Provincial boundaries are indicated on left figure, and district boundaries are marked on right figure.

mates. That is, container productivity predictions will be inaccurate if a significant proportion of the immature population fails to progress to adulthood.

Definition of the factors influencing *Ae. aegypti* emergence under field conditions may help to identify potential indicators that can be used in the prediction of adult productivity. Moreover, other characteristics of the aquatic environment, especially those related to nutrient availability, may provide further insight into the relationship between immature and emerging adult populations. Therefore, we examined the relationships between the abundance of III/IV instars and pupae and the emerging adult population for a series of water storage containers in southern Vietnam. We also examined potential associations between the size and/or nutrient content of IV instars and the resultant emergent adult population as a possible means of predicting emergence success.

Materials and Methods

Study Site and Container Selection. Field studies were undertaken in Tan Lan commune (10°31′37.51″N, 106°37′35.50″E), Long An province, located in the Mekong River Delta region in southern Vietnam (Fig. 1). Tan Lan (10,891 people, 2,470 households) is a rural community consisting of 10 hamlets interspersed throughout low-lying agricultural land primarily used for rice farming. There is no piped water or well water available to householders, and thus, they rely on harvesting of rainwater and storage of this in tanks (mainly 1,000-liter ferrocement cylindrical tanks) and jars (200-liter round jars). The area has a monsoonal climate, with wet season rains from April to October (mean daily temperature, 27.1°C; mean monthly rainfall, 242.1 mm) and a dry season between November and February (mean daily temperature, 26.7°C; mean monthly rainfall, 51.2 mm).

To select individual containers for inclusion in the study, a preliminary container survey was undertaken in November 2005. Households were randomly selected from a full list maintained by the local government. Householders were then invited to participate in the study, and if consent was given, a preliminary container survey was conducted to identify suitable containers for inclusion in the study. Round jars (200 liters) (Fig. 2) were selected for inclusion in the study, as these are the most common type of household water storage container in southern Vietnam (comprising 65% of all household water storage containers in Tan Lan commune; T.B.K., unpublished data). The presence of *Mesocyclops*, fish, or other predators was noted for each, and a 5-sweep net-sampling technique (Knox et al. 2007) was used to sample containers. Contents of nets were transferred to trays (43.2 × 53.3 × 6 cm; Ilford, Mobberley, Cheshire, United Kingdom). The

Fig. 2. Emergence trap for the collection of adult *Ae. aegypti* from 200-liter round jars at Tan Lan commune. A cylindrical netting bag was tied to a rigid, exterior frame, and was secured to the container with a drawstring. The trap interior was accessed through a sleeve (shown here on the left side of the trap), and contents were collected using a mechanical aspirator.

number of I/II instar Culicidae was counted together, and the number of III instar, IV instar, and pupal *Aedes* spp. was enumerated separately. Container and immature stage-specific conversion factors then were used to convert sample numbers to estimates of immature abundance (Knox et al. 2007).

Forty round jars (J1–J40) were selected for inclusion in the study based on an estimated population of ≥25 III/IV instar *Aedes* spp. per container. The 40 containers were located at 25 households. Each jar was then inspected for the presence of a cover, level of shading (partial or full), and cleanliness of water (clean, medium, dirty), and householders were asked the frequency of use of each (daily, less than daily). Water volume (percentage of fullness) was estimated for each jar, and water temperature was measured. Water analysis kits (Kyoritsu Chemical-Check Laboratory, Tokyo, Kanto, Japan) were used to measure chemical oxygen demand (COD), and the concentration of nitrites (NO_2) and nitrates (NO_3) was also measured as an indirect indicator of organic compounds.

Emergence Trapping and Sampling of Immatures. An emergence trap was placed atop each of the 40 jars (Fig. 2), and householders were requested to leave the traps undisturbed for the duration of the study. The trap consisted of a white mesh (0.01 mm aperture) cylindrical-shaped bag (38 cm diameter × 40 cm height) attached to an exterior metal frame (40 cm diameter × 30 cm height) and secured to the container lip exterior by a drawstring. Emerged adults were collected from traps between 0700 h and 1100 h each day using a mechanical aspirator. Water volume (percentage of fullness) was noted for each jar on each day of the study, and water surfaces were examined (through emergence traps) for dead adults. Collected mosquitoes were transported to the field laboratory in aspirator tubes, where they were killed using ethyl acetate and stored at 4°C until further processing.

Five-sweep net sampling was conducted every 3 d (days 3, 6, 9, 12, 15) after the initial sampling (day 0), and the numbers of I/II instar Culicidae and III instar, IV instar, and pupal *Aedes* were recorded. During initial and subsequent sampling, subsamples of III and/or IV instars ($n = 5$) were randomly selected from net yields if there were sufficient numbers of the stage present (i.e., an estimated population of ≥25). After final net sampling, up to five III instars and five IV instars were subsampled from each container. Subsampled immatures were transferred to 5-ml tubes and were transported to the field laboratory, where they were identified to species, measured for head capsule width, and then stored for further evaluation of nutrient content. Those immatures that were collected and enumerated for population estimates, but were not retained for nutritional analyses, were returned to the container from which they were sampled.

Rainfall and daily temperature data for Long An city (≈40 km from Tan Lan commune) were obtained for the period 1 October to 30 November 2005 from the Vietnamese Bureau of Meteorology.

Analysis of the Relationships Between Immature Abundance and Adult Emergence. The number of *Ae. aegypti* III and IV instars was summed for each sample. The population of III/IV instars and pupae was estimated for each jar at each sampling event by multiplying sweep net-sampling yields by the relevant conversion factors (2.85 for III/IV instars and 3.19 for pupae; Knox et al. 2007). To investigate the relationship between immature abundance and the adult population emerging over successive time periods, linear regressions were conducted: the estimated number of III/IV instars at initial sampling (less any immatures that were removed for nutritional assays) was compared with the number of adults emerging from 48 to 72, 120, 168, 240, and 360 h, and the estimated number of pupae at initial sampling was compared with the number of adults emerging from 0 to 24, 48, 72, 96, and 120 h, for each jar.

To provide an indication of the relative number of immatures progressing to adulthood, quotients were calculated: larval emergence quotient (LEQ) was the number of adults emerging 48–240 h after sampling/estimated III/IV instar population (less any immatures that were removed for nutritional assays) × 100%; pupal emergence quotient (PEQ) was the number of adults emerging 0–48 h after sampling/estimated pu-

pal population (minus subsamples) × 100%. To allow for the effect of sampling variability, 95% confidence intervals for the sampled numbers of III/IV instars and pupae for each container were calculated using the Poisson distribution (at $P = 0.05$). The upper and lower 95% confidence limits (CL) were multiplied by III/IV instar and pupal conversion factors to derive 95% confidence intervals for estimated numbers of III/IV instars and pupae. Minimum and maximum LEQ and PEQ were then calculated, and these were used as indicators of particularly low or high adult emergence rates with respect to immature abundance. A LEQ_{MAX} of ≤25% was assigned as the cutoff for adult underemergence relative to III/IV instar abundance, and a PEQ_{MAX} of ≤50% was assigned for adult underemergence with respect to pupal abundance. These values were based on laboratory-derived data on the differing survivorships to adulthood, of pupae and III/IV instars reared under low nutritional conditions (0.03–0.1 mg food per larva per day) (T.B.K., unpublished data). Note that emergence quotients >100% were possible if the number of adults emerging over the specified time period exceeded the number of immatures estimated on the reference day. The uncertainty in the estimated numbers of immatures as a result of net sampling may have resulted in an underestimate of the numbers of immatures in the container, and hence, the numbers of emerging adults could be higher than the estimated numbers of immatures (emergence quotients >100%).

Female Wing Lengths, IV Instar Head Capsule Widths, and Lipid, Sugar, and Glycogen Content. All field-collected immatures and adults were identified to species, and adult *Ae. aegypti* were examined for sex. Wing lengths of up to five females collected from each container on each day and head capsule widths of subsampled III and IV instars ($n = 304$) were measured using an Olympus CH-2 compound microscope (Olympus, Shinjuku-ku, Tokyo, Japan). Fourth instar specimens for lipid, free sugar, and glycogen analysis were placed individually in 100 μl of 70% ethanol and then preserved to prevent enzymatic degradation of carbohydrates by heating tubes at 100°C for 3 min in a QBD-2 heating block (Grant Scientific, Shepreth, Cambridgeshire, United Kingdom), and were stored at −20°C before analysis using a modification of methods described by van Handel and Day (1988). Sodium sulfate (0.2 ml of 2% solution) was added to tubes containing preserved larvae, and these were ground using micropestles and a cordless motor (Quantum Scientific, Brisbane, Queensland, Australia). A total of 1 ml of chloroform-methanol (1:2) was added, and specimens were mixed thoroughly using a Solid State Control Vortex Mixer (Ratek Instruments, Boronia, Victoria, Australia) and then centrifuged at 5,000 rpm for 5 min at 25°C in a 2K15 laboratory centrifuge (Sigma Laborzentrifugen, Osterode am Harz, Germany). The supernatant was taken up using a 2.0-ml graduated syringe, and was distributed equally between two 10-ml glass culture tubes. The contents of the first tube were analyzed for lipids using the vanillin-phosphoric acid method (van Handel 1985a). Briefly, the tube was heated to 100°C until all of the solvent was dissolved, and then 0.2 ml of sulfuric acid was added and the tube was heated for an additional 10 min at 100°C. After cooling, vanillin-phosphoric acid reagent was added to a total of 5 ml. Tube contents were mixed thoroughly, and 0.2 ml was pipetted in replicates of four into a flat-bottomed 96-well plate. Absorbances were read at 525 nm between 5 and 25 min after mixing using a VERSAmax tunable microplate reader (Molecular Devices, Sunnyvale, CA). The second tube was processed for sugar content using the hot anthrone method (van Handel 1985b). Briefly, the tube was heated to 100°C until all but ≈0.1 ml of the solvent had evaporated. Anthrone reagent was added to a total of 5 ml, and samples were mixed thoroughly and then heated at 100°C for 17 min. After cooling and mixing, replicates were pipetted into 96-well plates, as above, with absorbances read at 625 nm. The precipitate remaining in the original microcentrifuge tube was extracted in anthrone reagent, added to a third tube, and processed for glycogen content as for the sugar assays.

Standard curves were constructed in triplicate using soybean oil for vanillin-phosphoric acid assays (0, 5, 10, 15, 20, 25, 50, 100, and 200 μg) and D-glucose for hot anthrone assays (0, 5, 10, 15, 20, 25, 50, 100, 150 μg). The soybean standard curve equation was used to calculate lipid content, and the D-glucose standard curve equation was used to calculate free sugar and glycogen content for each IV instar specimen. Positive controls (20 and 100 μg of soybean oil or D-glucose) were run with each batch of assays to ensure consistency, and a negative control (i.e., all reagents, but no larval sample) was included as a blank against which to read all values.

Results

All collected *Aedes* specimens (III and IV instars and adults) were identified as *Ae. aegypti*. Some *Anopheles* and *Culex* spp. were also collected, although these were not enumerated. A total of 2,982 adult *Ae. aegypti* was collected from 39 of the jars over 18 d, with one jar (J7) producing no adults. The greatest total number of adults collected from any jar by emergence trapping was 335 (J9); the greatest number collected from a single jar within a 24-h period was 99 adults (J26). A total of 11,575 *Ae. aegypti* immatures was sampled from the 40 jars via 5-sweep netting. Total estimated immature populations at initial sampling were 1,887 III instars, 2,841 IV instars, and 1,219 pupae; the estimated number of III/IV instars and pupae per container ranged from 14 to 770 and 0 to 188, respectively (Table 1). Four jars dried out (J4, J22, J32, and J40 on days 18, 18, 11, and 13, respectively), and for two jars householders removed the emergence trap and added water (J9 and J10 both on day 12) before the conclusion of the study; data collected for these six jars after dryness/refilling were disregarded in subsequent analyses.

Estimates of immature populations and emergent adult populations were generally positively skewed, so

Table 1. Abundance and physical characteristics of *Ae. aegypti* populations in 40 × 200-liter round earthenware jars at Tan Lan commune in southern Vietnam

Jar	No. at initial sample[a]		Total[b]	Emergence success		Mean size		Mean nutrient content		
	III/IV instars	Pupae	Adults	LEQ[c]	PEQ[d]	Female wing lengths	Head capsule widths[e]	Lipid[e]	Free sugar[e]	Glycogen[e]
J1	22.8	19.1	100	78.1	52.2	2.509				
J2	145.4	0	73	24.4		2.481	0.862	0.249	0.193	0.672
J3	31.4	3.2	41	122.7	156.7	2.476				
J4[f]	14.3	9.6	19	112.3	31.3	2.630				
J5	199.5	76.6	227	61.2	130.0	2.312	0.902	0.262	0.126	0.526
J6	225.2	3.2	8	2.4	0	1.967	0.878	0.158	0.234	0.368
J7	85.5	0	0	0			0.842	0.118	0.157	0.284
J8	142.5	188.2	179	2.4	36.0	2.624	0.877	0.413	0.240	0.610
J9[g]	356.3	89.3	335		66.4	2.465	0.824	0.676	0.268	0.806
J10[g]	134.0	28.7	129		189.8	2.569	0.890	0.783	0.227	0.918
J11	584.3	0	301	48.6		2.332	0.863	0.533	0.271	0.912
J12	28.5	0	19	63.8		2.476	0.798	0.320	0.232	0.757
J13	59.9	19.1	8	6.0	26.1	2.273	0.808	0.087	0.287	0.155
J14	362.0	102.1	176	33.2	55.6	2.126	0.874	0.228	0.219	0.735
J15	191.0	9.6	1	0	10.4		0.892	0.093	0.209	0.281
J16	57.0	38.3	58	116.7	23.5	2.219	0.824	0.186	0.121	0.473
J17	82.7	3.2	9	13.3	0	1.883	0.795	0.113	0.133	0.350
J18	74.1	9.6	34	34.3	10.4	2.066	0.765	0.198	0.238	0.544
J19	39.9	0	6	17.2		1.880	0.838	0.093	0.119	0.215
J20	20.0	12.8	41	125.3	31.3	2.547				
J21	139.7	19.1	32	9.3	15.7	2.295	0.886	0.110	0.085	0.111
J22[f]	77.0	79.8	154	143.2	68.2	2.409	0.877	0.376	0.250	0.963
J23	14.3	86.1	99	610.5	14.8	2.430				
J24	71.3	86.1	114	111.7	35.7	2.646	0.930	0.590	0.271	1.591
J25	105.5	0	18	14.4		2.140	0.857	0.247	0.133	0.600
J26	136.8	82.9	236	93.3	141.1	2.243	0.893	0.367	0.211	0.617
J27	22.8	0	13	22.5		2.348				
J28	20.0	6.4	6	15.0	47.0	2.450				
J29	39.9	0	42	100.3		2.776	0.948	0.458	0.346	1.153
J30	114.0	28.7	85	58.7	62.7	2.589	0.907	0.711	0.209	1.244
J31	99.8	6.4	72	68.6	78.4	2.364	0.868	0.199	0.196	0.558
J32[f]	48.5	102.1	178		122.6	2.524	0.868	0.350	0.166	0.721
J33	20.0	3.2	55	45.7	0	2.383	0.804	0.129	0.099	0.306
J34	34.2	0	3	2.9		2.145				
J35	31.4	0	20	93.7		2.113	0.824	0.167	0.219	0.568
J36	17.1	16.0	18	154.9	31.3	2.397				
J37	37.1	6.4	16	31.2	78.4	2.193	0.862	0.167	0.205	0.573
J38	22.8	3.2	1	5.6	0					
J39	51.3	0	5	2.2		1.900	0.832	0.042	0.064	0.056
J40[f]	769.5	79.8	51		40.1	2.251	0.888	0.227	0.145	0.489

[a] Estimated number using 5-sweep net sampling and conversion factors from Knox et al. (2007).
[b] Total number collected over duration of study using emergence trapping.
[c] Adults emerging 48–240 h after sampling/estimated III/IV instar abundance (minus subsamples) × 100%.
[d] Adults emerging 0–48 h after sampling/estimated pupal abundance (minus subsamples) × 100%.
[e] For IV instars subsampled during initial 5-sweep netting.
[f] Water in jar dried out prior to end of study.
[g] Water was refilled by householder prior to end of study.

all data were transformed using the following: population (transformed) = $\log_{10}$ ([estimated population] + 1). There were significant, but weak linear relationships between *Ae. aegypti* III/IV instar populations estimated via 5-sweep sampling on the initial day of sampling and the number of adults emerging 48–72, 48–120, and 48–168 h thereafter ($P = 0.026–0.037$) (Fig. 3). However, there was no significant relationship with adults emerging 48–240 and 48–360 h thereafter ($P = 0.067$ and 0.110, respectively). All of the 40 jars held *Ae. aegypti* III/IV instars and 39 produced at least one adult over the 18-d study period. However, the presence of III/IV instars was not necessarily a definitive indicator of adult productivity over specified time periods. For example, J7 and J15 contained an estimated 76 and 181 III/IV instars (after subsamples were extracted), respectively, but produced no adults from 48 to 360 h after sampling.

Pupal abundance estimates were more closely related to emerging adult populations than were III/IV instar estimates (all $P < 0.001$; Fig. 3); the strongest relationship was for adults produced from 0 to 48 h ($R^2 = 0.792$). Initial sampling detected no pupae in 11 of the 40 jars. Although 10 of these containers produced at least one adult over the study duration, pupal negativity during sampling was a good predictor of no or low adult production over specified time periods. That is, adult production remained low (≤0.5 adults per day) for all but one jar (J11) up to 96 h after initial sampling. Conversely, the detection of pupae was a

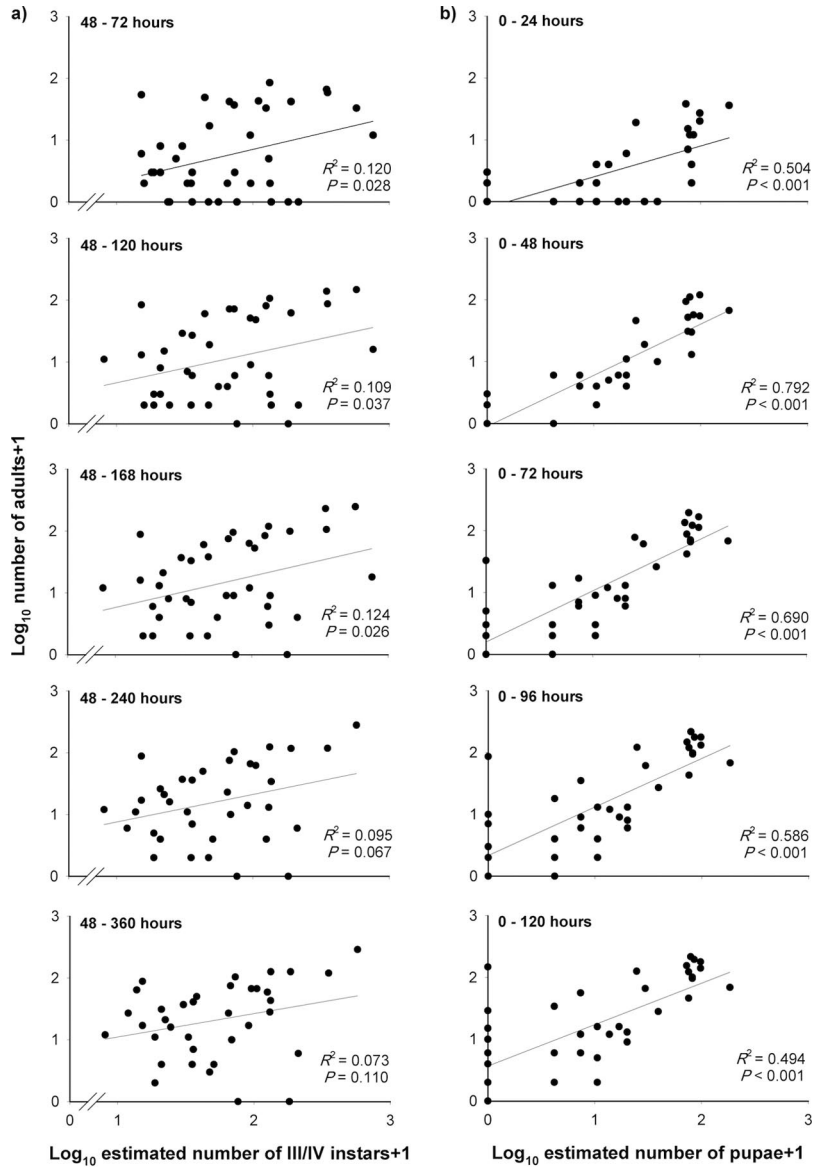

Fig. 3. Relationship between *Ae. aegypti* III/IV instar (*a*) and pupal abundance (*b*) (estimated on initial day by 5-sweep net sampling) and emergent adult population (determined by emergence trapping) over the postsampling duration specified, for 40 × 200-liter round jars at Tan Lan commune. All data were log(+1) transformed; regression lines and statistics are indicated.

good indicator of subsequent adult emergence; by 48 h after sampling, 24 of the 29 jars containing ≥1 pupa had produced ≥1 adult, and by 120 h all 29 had produced at least one adult.

For further analyses of emerging adult populations in relation to III/IV instar abundance, adult data for 48–240 h were selected. This was based on laboratory development times of 9.5 d from III instars to adult at ≥20°C (Christophers 1960, Rueda et al. 1990, Tun-Lin et al. 2000) and 11.9 d in water jars in the field (Southwood et al. 1972). Calculated LEQ ranged between 0 and 610.5% (95% CI: 0–211.9%), indicating that the number of adults produced from a jar was between 0 and 6.1 times the estimated number of III/IV instars in the jar at initial sampling. There was no correlation between LEQ and the abundance of III/IV instars at initial sampling ($r = -0.190$, $n = 36$, $P = 0.268$). The median LEQ_{min} for field jars was 27.2% (95% CI: 0–142.8%), and the median LEQ_{max} was 60.3% (95% CI: 0–815.8%). For example, for J21, we collected 49 III/IV instars during the initial sampling period, and the 95% CI for this estimate, based on the assumption that sampled numbers follow a Poisson distribution with $mu = 49$, was 38–60 III/IV instars. This corre-

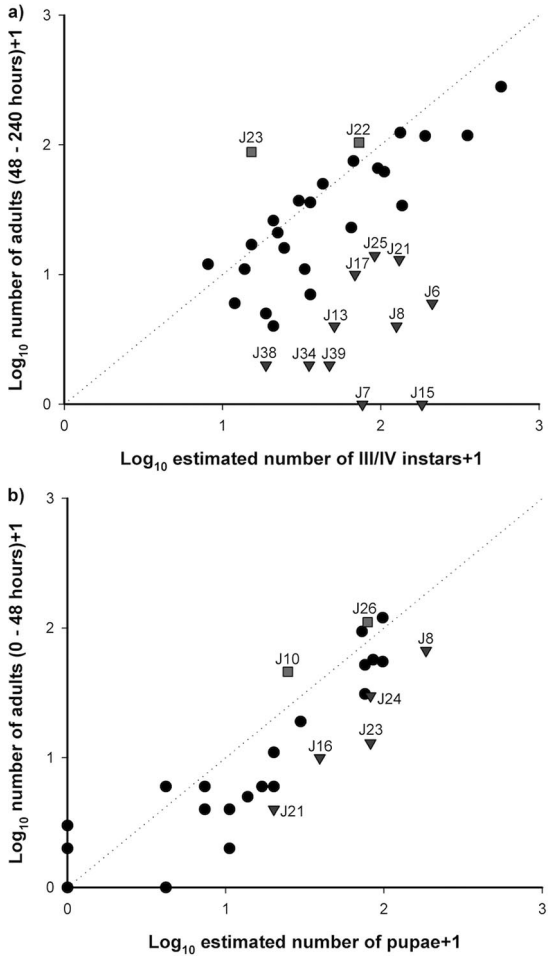

Fig. 4. Number of *Ae. aegypti* emerging from 200-liter round jars versus estimated number of III/IV instars (*a*; n = 36 jars) and pupae (*b*; n = 40 jars) at Tan Lan commune. Additional symbols (and codes) indicate jar populations exhibiting significant underemergence (▼) or overemergence (■) based on adult productivity versus III/IV instar abundance ($LEQ_{max} \leq 25\%$ and $LEQ_{min} \geq 100\%$, respectively) or pupal abundance ($PEQ_{max} \leq 50\%$ and $PEQ_{min} \geq 100\%$, respectively). All data were log(+1) transformed; dotted lines show an emergence quotient of 100%.

sponded to an estimated population of 108.3–171.0 III/IV instars. From this, the calculated LEQ_{MAX} and LEQ_{MIN} were 12.2 and 7.5%, respectively. Thus, ≈7.5–12.2% of the estimated III/IV instar population from J21 emerged as adults from 48 to 240 h after sampling. Of the 36 containers sampled for larvae, 11 were found to have produced significantly fewer than expected numbers of adults ($LEQ_{MAX} \leq 25\%$) (Fig. 4).

For further analyses of emerging adult populations versus pupal abundance, adult data from 0 to 48 h after sampling were selected. Calculated PEQ ranged between 0 and 189.8% (95% CI: 0–166.7%), indicating that the number of adults produced from a jar was between 0 and 1.9 times the estimated number of pupae in the jar at initial sampling. There was no correlation between PEQ and the abundance of pupae ($r = 0.191$, $n = 29$, $P = 0.321$) in field jars. The median PEQ_{MIN} was 26.0% (95% CI: 0–103.5%), and the median PEQ_{MAX} was 83.1% (95% CI: 28.5–284.2%), respectively. Of the 40 containers sampled for pupae, five were found to have produced significantly fewer than expected numbers of adults ($PEQ_{MAX} \leq 50\%$) (Fig. 4).

Adult Size Versus IV Instar Size, Nutrient Content, and Habitat Characteristics. Wing lengths, head capsule widths, and free sugar and glycogen content were associated with the COD of the water and the absence of a cover on the jar ($P < 0.05$ for all; Table 2). That is, larger and better-nourished *Ae. aegypti* were collected from jars that were uncovered and contained water with a higher COD. Water volume was negatively related to IV instar head capsule widths and free sugar content, such that fuller jars produced smaller IV instars with lower sugar content. Glycogen content of IV instars was negatively related to temperature ($P = 0.008$), but lipid content was not significantly related to any of the measured water or container characteristics ($P > 0.05$ for all). Head capsule widths of IV instar *Ae. aegypti* increased with NO_3 levels ($P < 0.001$) and were larger in fully shaded containers ($P = 0.037$) and in containers harboring other Culicidae ($P = 0.029$). However, the presence of potential predators and competitors did not have a significant impact on the size and/or nutrient content of emerging adults or immatures (all $P > 0.05$).

For relationships between habitat parameters related to nutrient availability, there were no significant correlations between NO_2, NO_3, and COD (all $P > 0.05$), although NO_3 and COD were related to water volume. COD was significantly higher for uncovered compared with covered containers ($T = 266.0$, $n = 18,22$, $P = 0.005$); however, there was no significant difference in nitrites ($T = 325.5$, $n = 18,22$, $P = 0.239$), nitrates ($T = 317.0$, $n = 18,22$, $P = 0.159$), temperature ($T = 431.5$, $n = 18,22$, $P = 0.091$), or fullness ($T = 533.5$, $n = 18,22$, $P < 0.001$) between covered and uncovered containers.

Adult Emergence Versus Adult Size, IV Instar Size, and Nutrient Content. LEQ and PEQ were both positively correlated with IV instar head capsule widths ($P = 0.009$ and 0.011, respectively) (Table 2); that is, the proportion of IV instars and pupae emerging to adults was associated with the mean size of IV instar *Ae. aegypti* from the same jar. Moreover, mean head capsule widths were significantly smaller for jars exhibiting significant under- versus regular emergence ($F_{1,24} = 5.257$, $P = 0.031$). However, linear regressions of LEQ and PEQ with head capsule widths were not significant ($P = 0.176$ and 0.054, respectively) (Fig. 5). The mean lipid and free sugar content of IV instars was also correlated with LEQ ($P = 0.017$, 0.001, respectively), although glycogen content was not ($P = 0.179$) (Table 2); neither lipid, sugar, nor glycogen content was correlated with PEQ ($P > 0.05$ for all). Lower lipid and free sugar content was also associated with underemergence in relation to III/IV instar pop-

Table 2. Summary statistics for correlations and associations of *Ae. aegypti* larval and pupal emergence quotients and sex ratios for 40 × 200-liter round jars at Tan Lan commune, and the mean size and nutritional content of adults and immatures (by Pearson's correlation), water measurements (by Pearson's correlation), and container characteristics (by analysis of variance) for each jar

Parameters	Larval emergence quotient			Pupal emergence quotient		
Adult and IV instar factors	*r*	*n*	*P*	*r*	*n*	*P*
Mean adult female wing length	0.445	24	0.029*	0.417	20	0.068
Mean IV instar head capsule width	0.503	26	0.009**	0.543	21	0.011*
Mean IV instar (log) lipid content	0.465	26	0.017*	0.213	21	0.355
Mean IV instar (log) free sugar content	0.613	26	0.001**	0.316	21	0.163
Mean IV instar (log) glycogen content	0.272	26	0.179	0.425	21	0.055
Water measurements						
Fullness (%)	−0.315	36	0.061	−0.545	29	0.002**
Temperature	0.150	36	0.381	−0.491	29	0.007**
Nitrites (NO_2)	0.374	36	0.025*	0.044	29	0.821
Nitrates (NO_3)	−0.053	36	0.758	0.472	29	0.010*
(log) COD	0.564	36	<0.001**	0.274	29	0.150
Container characteristics	*F*	df	*P*	*F*	df	*P*
Shade status	0.633	1, 34	0.432	1.666	1, 27	0.208
Lid +/−	4.401	1, 34	0.043*	6.313	1, 27	0.018*
Frequency of use	1.061	1, 34	0.310	1.107	1, 27	0.302
Water cleanliness	0.352	2, 33	0.706	3.870	2, 26	0.034*
Copepod +/−	0.886	1, 34	0.353	0.669	1, 27	0.420
Micronecta spp. +/−	0.115	1, 34	0.737	0.761	1, 27	0.391
Ostracod +/−	0.310	1, 34	0.581	0.478	1, 27	0.495
Other Culicidae +/−	0.243	1, 34	0.625	1.863	1, 27	0.184

Asterisks indicate significant differences at the 0.05 level (*) or the 0.01 level (**) (by Pearson's correlation or analysis of variance).

ulations ($F_{1,24} = 5.773$, $P = 0.024$, and $F_{1,24} = 8.940$, $P = 0.006$, respectively). Furthermore, the linear relationship between LEQ and both lipid and free sugar content was statistically significant ($P = 0.009$ and 0.017, respectively) (Fig. 5), with the strongest relationship for lipids. For example, mean lipids of ≥0.20 mg/IV instar were associated with positive emergence, and, with one exception, mean lipids of ≥0.30 mg/IV instar were associated with LEQ of ≥40%. However, for jars for which mean lipid content was ≤0.20 mg/IV instar, LEQ varied from 0 to 116.7% (mean ± SD of 41.5 ± 40.0%). Such large variation may mean that although high lipids were a good indicator of a high emergence success rate, predictive utility was poor when lipid levels were low.

The concentration of NO_2 and the COD of the water in jars was significantly positively correlated with LEQ ($P = 0.025$ and <0.001, respectively) (Table 2), such that the emergence success of III/IV instars increased as the level of nitrites and the COD increased. The COD also varied significantly between jars classified as under- or regular producers such that higher levels were associated with greater emergence success from the late instars ($F_{1,34} = 4.391$, $P = 0.044$). Water fullness, temperature, and NO_3 concentration were correlated with PEQ ($P = 0.016$, 0.007, and 0.010, respectively), such that emergence from the pupal stadium increased as the level of nitrates increased and as water volume and temperature decreased. Furthermore, jars exhibiting under- and regular emergence differed significantly in fullness such that underemergence was associated with a greater volume of water ($F_{1,34} = 6.474$, $P = 0.016$).

Of the container characteristics recorded, the presence of a cover had a significant effect on emergence from both larval and pupal stages such that emergence rates were higher for uncovered jars (Table 2). Mean LEQ was 33.1 ± 38.0 versus 102.7 ± 135.4, and PEQ was 21.5 ± 26.6 versus 68.1 ± 52.4 for covered and uncovered jars, respectively. Pupal emergence was also influenced by the cleanliness of water such that emergence increased with dirtiness; PEQ was 24.4 ± 21.0 versus 31.1 ± 40.5 versus 76.5 ± 52.7 for clean, medium, and dirty water, respectively. However, there was no significant difference in lid status, water cleanliness, or any of the other container characteristics between jars exhibiting under- and regular emergence ($P > 0.05$). The presence of potential predators and competitors of *Ae. aegypti* larvae (i.e., copepods, *Micronecta* spp., or ostracods) did not affect LEQ or PEQ ($P > 0.05$ for all), and there was no significant difference in the presence or absence of these for containers exhibiting under- or regular emergence of *Ae. aegypti* ($P > 0.05$ for all).

Discussion

Our assessments of the relationships between *Ae. aegypti* immature abundance and adult mosquito emergence for water storage jars have clearly highlighted the superiority of pupal counts over III/IV instar counts as direct indicators of adult mosquito productivity. Relationships between the estimated abundance of III/IV instars and the cumulative number of adults emerging over five selected time periods were poor ($R^2 = 0.07$–0.12; Fig. 3), and emergence

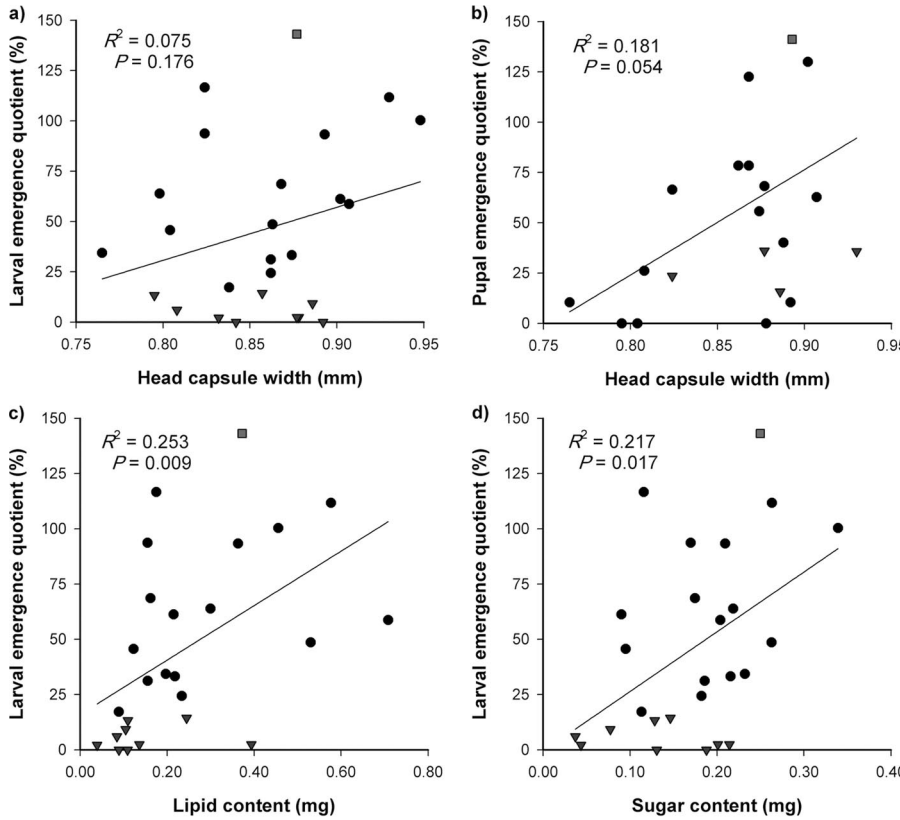

Fig. 5. Larval or pupal emergence quotients (LEQ or PEQ) versus mean IV instar head capsule widths (*a*, *b*), lipid content (*c*), and free sugar content (*d*) for *Ae. aegypti* from 200-liter round jars at Tan Lan commune. Additional symbols indicate jar populations exhibiting significant underemergence (▼) or overemergence (■) based on adult productivity versus III/IV instar abundance ($LEQ_{MAX} \leq 25\%$ and $LEQ_{MIN} \geq 100\%$, respectively) or pupal abundance ($PEQ_{MAX} \leq 50\%$ and $PEQ_{MIN} \geq 100\%$, respectively). Regression lines and statistics are indicated.

success from III/IV instars varied widely between jars (0–610%). Clearly, predictions based solely on III/IV instar estimates would be inaccurate and would result in an overestimation of adult productivity. Estimated pupal abundance provided a better indication of emerging adult populations, particularly over the 48-h period subsequent to initial immature sampling ($R^2 = 0.79$).

The unknown developmental history of immatures (e.g., duration already spent in the instar at the commencement of the study), the existence of multiple cohorts, intraspecific competition, and stochastic sampling effects all contributed to the poor relationship between III/IV instar abundance and adult productivity. As direct counts of immatures from 200-liter containers were not feasible, and efforts to remove and count all the immatures probably would have resulted in significant disruption to the containers, we chose to use an established quantitative net sampling method and stage-specific conversion factors to estimate standing crops of immatures (Knox et al. 2007). As these estimates were not without error, we incorporated the variability in the estimated numbers of immatures into our LEQ and PEQ calculations. Using the lower and upper 95% CL for the estimated numbers of immatures in each container, we were able to examine the upper (LEQ_{MAX} and PEQ_{MAX}) and lower (LEQ_{MIN} and PEQ_{MIN}) limits, respectively, for the proportions of the standing crops of immatures that emerged as adults over different time periods. For III/IV instar abundance, we interpreted a LEQ_{MAX} of <25% to mean that the estimated numbers of III/IV instars overrepresented the relative productivity of the container in terms of adult mosquito emergence. We calculated LEQ_{MAX} for 36 jars, and 31% were found to have values <25%, and meant that these containers produced 75% fewer adults than expected, based on the assumption that there was no delayed immature development beyond 18-d duration, or immature mortality. In comparison, when we calculated the PEQ_{MAX} for pupae, with a less conservative cutoff of 50%, only 12.5% (five) of containers produced 50% fewer adults than expected. These relatively simple comparisons of the proportions of immatures that emerged as adults highlighted the potential problems associated with extrapolation of adult numbers based on immature abundance.

As we purposefully selected III/IV positive containers (e.g., ≥25 III/IV instars) for inclusion in this study, there may have been a bias in favor of containers with

higher numbers of immatures, with possible inclusion of cohorts that were subject to greater intraspecific competition effects. Notwithstanding this, we are aware of the difficulties in sampling immatures from containers, particularly at relatively low densities (Knox et al. 2007), and therefore, it is more than likely that adult productivity will be underestimated when immature densities are low. For example, for net sampling from 200-liter round jars, we previously calculated 50% sensitivity levels for detection of III/IV instars and pupae, as two and seven immatures per jar, respectively. For pupae at least, with a higher threshold for detection and lower overall abundance compared with III/IV instars, there may be significant underestimation of productivity when densities are low. As the sensitivity of sampling pupae from larger containers (1000-liter cylindrical tanks) is relatively low (50% detection threshold of 15 pupae per container), then the underestimation of adult productivity based on pupal abundance will be greater in areas where large containers are more prevalent. Conversely, it is likely that the accuracy of estimates will be greater where containers are smaller. Therefore, although there was a strong relationship between pupal abundance and adult productivity, practical limitations associated with enumeration of pupae should also be taken into account.

Explanatory variables associated with low LEQ were mainly related to low availability of nutrients. Uncovered containers, which held water with higher amounts of organic compounds (as indicated by the COD) and thus were presumably more exposed to nutrient sources (e.g., falling leaves), were associated with higher emergence rates from both III/IV instar and pupal stadia than were covered containers. Strickman and Kittayapong (2003) found that larger adults were produced from uncovered containers, as well as from containers exhibiting other qualities favoring nutrient availability. In the current study, nitrate concentration and COD were directly related to larval emergence quotients, with pupal emergence quotients affected by nitrates, the cleanliness of water, but also by the water volume and temperature. Although the proportion of III/IV instar populations emerging over 48–240 h and pupal populations emerging over 0–48 h was significantly correlated with head capsule widths, linear relationships were weak, and indicated that head capsule size was unlikely to be a useful indicator of emergence success.

The lipid content of *Ae. aegypti* IV instars, and to a lesser extent, the free sugar content, were linearly related to emergence success from III/IV instars for field populations. However, where lipid levels were low, predictions of emergence success based on head capsule widths would be less reliable. As such, it is unlikely that the assessed physical characteristics of *Ae. aegypti* immatures would be useful indicators of emergence success from immature stadia.

Other field investigations have noted associations between factors that can influence the availability of food, such as container size, location, covered status, and water-filling method, and the abundance of *Ae. aegypti* (Shannon and Putnam 1934, Tun-Lin et al. 2000, Strickman and Kittayapong 2003, Schneider et al. 2004, Vezzani et al. 2005, Barrera et al. 2006). Food source/quality is also known to affect adult *Ae. aegypti* size and nutrient content (Shannon and Putnam 1934, Scriber and Slansky 1981, Timmerman and Briegel 1996, Barrera 1996, Arrivillaga and Barrera 2004). Barrera et al. (2006) found that the pupal productivity and biomass of emerging females varied between containers based on the presence of litter of different tree species, and numerous studies have investigated specific dietary components required for the development of mosquitoes (Merritt et al. 1992, Canavoso et al. 2001). Notwithstanding these findings, *Ae. aegypti* have also been shown to exhibit an exceptional ability to cope with food of low nutritional value (Arrivillaga and Barrera 2004). Others have concluded that the production of a larval growth retardant in response to limited nutrient availability (Moore and Fisher 1969, Moore and Whitacre 1972) or crowding (Dye 1984) were the main intraspecific regulators of *Ae. aegypti* populations. In the current study, estimated III/IV instar and pupal densities were not related to emergence success, although pupal success was related to water level and to the size and free sugar and glycogen content of IV instars. Overall, with the exception of the incorporation of occasional measurements of wing lengths of emergent *Ae. aegypti* as a marker of higher vectorial capacity (Strickman and Kittayapong 2003), none of these factors relating to the immature environment have been incorporated into operational programs to aid the interpretation of immature surveillance data.

This study has demonstrated some clear advantages of pupal surveillance over surveillance of III/IV instars for estimation of adult mosquito productivity. However, effective utilization of pupal survey methodologies to prioritize control activities or to parameterize transmission models to prescribe epidemiological risk requires the consideration of the practical limitations associated with enumeration of pupae (Focks and Alexander 2006; Knox et al. 2007), which are often present in low densities. As a result of lower numbers and reduced sampling sensitivity, pupal surveys may be acceptable for definition of key containers, but will underestimate the extent of container positivity (Knox et al. 2007). Thus, we believe that it is important that operational surveillance activities also consider surveillance of larvae if resources permit.

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