Rapid Estimation of *Aedes aegypti* Population Size Using Simulation Modeling, with a Novel Approach to Calibration and Field Validation

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ABSTRACT New approaches for control of the dengue vector *Aedes aegypti* (L.) are being developed, including the potential introduction of life-shortening symbiont bacteria into field populations and the release of transgenic strains with reduced vector competency. With these new approaches comes the need for rapid estimations of existing field population size. Here, we describe the use of simulation modeling with container-inhabiting mosquito simulation (CIMSiM) for estimation of *Ae. aegypti* pupal crop size in north Queensland, Australia. CIMSiM was calibrated for local conditions by deploying "sentinel key containers" (tire, 2-liter plastic bucket, 0.6-liter pot plant base, and tarpaulin indentation) in which water flux and pupal productivity were studied for 72 d. Iterative adjustment of CIMSiM parameters was used to fit model outputs to match that of sentinel key containers. This calibrated model was then used in a blind field validation, in which breeding container and local meteorological data were used to populate CIMSiM, and model outputs were compared with a field pupal survey. Actual pupae per ha during two 10-d periods in 2007 fell within 95% confidence intervals of simulated pupal crop estimates made by 10 replicate simulations in CIMSiM, thus providing a successful field validation. Although the stochasticity of the field environment can never be wholly simulated, CIMSiM can provide field-validated estimates of pupal crop in a timely manner by using simple container surveys.

KEY WORDS *Aedes aegypti*, CIMSiM, modeling, population, pupal crop, simulation

Aedes aegypti (L.) is a prominent vector of dengue viruses (family *Flaviviridae*, genus *Flavivirus*, DENV) throughout tropical regions. The resurgence of DENV worldwide (Farrar et al. 2007) is necessitating novel approaches to improve surveillance and control of its main vectors, which have strong associations with human habitation. New approaches currently being developed include the introduction of life-shortening symbiont *Wolbachia* bacteria into field populations (Sinkins 2004; www.mosquitoage.org) and transgenic mosquito strains that have attenuated DENV transmission competence (James 2005; www.gcgh.org/Projects/ControlInsectVectors/GeneticStrategy/).

For the potential release of *Wolbachia*-infected or transgenic *Ae. aegypti*, knowledge of wild population size, life-stage population structure, and adult female age is essential for devising release strategies. Some of these data are obtainable from house-to-house surveys of immature life stages (larvae and pupae) (Focks 2003), and to a lesser extent from adult collection by aspiration (Clark et al. 1994) or trapping (Williams et al. 2006). However, collection of population data by these methods has large labor and time requirements, such that appropriate timeliness would be unachievable. Clearly, rapid population assessment methods need to be developed.

Life-table simulation models provide a plausible method for rapid estimation of *Ae. aegypti* population size and structure, and for prediction about future population size. A weather-driven container-inhabiting mosquito simulation model (CIMSiM) (Focks et al. 1993a,b) has already been developed for container-breeding mosquitoes such as *Ae. aegypti.* CIMSiM provides the framework describing the interaction between *Ae. aegypti* and its environment, by using laboratory and field-derived relationships describing growth, development, and behavior. This is achieved through the creation of virtual breeding habitats (i.e., containers) in which water filling and emptying rates (water flux), size, thermal dynamics, and larval food input are set. The input of meteorological data (rainfall, humidity, and temperature), breeding container type and density data, and human population data allows the generation of life-table simulations of *Ae. aegypti* populations (Focks et al. 1993a,b). In theory, a field calibrated version of this model could be used to estimate population size in a discrete area, using

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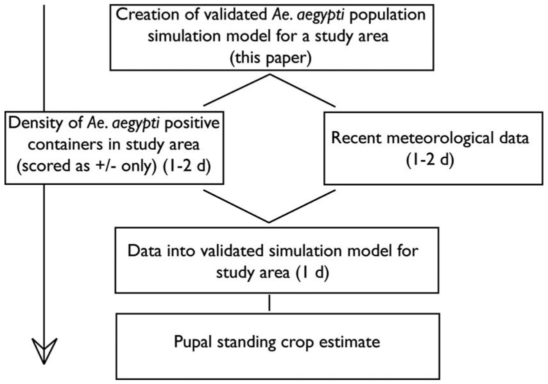

Fig. 1. Flowchart of steps and time required for rapid *Ae. aegypti* population size estimation by using CIMSiM.

only a brief breeding container survey (1–2 d) and readily available meteorological data (Fig. 1).

The ecology of *Ae. aegypti* is regionally variable, especially with respect to the nature of larval habitat (i.e., water-filled containers) (e.g., Chadee et al. 1984; Nam et al. 1998). Therefore, the CIMSiM model needs to be calibrated for application in specific areas. This process involves matching field measures of *Ae. aegypti* productivity in specific containers to that simulated by CIMSiM by iteratively adjusting the model to fit (Focks et al. 1993b). This requires detailed knowledge about water flux and pupal productivity of breeding containers responsible for the majority of *Ae. aegypti* production, so-called "key containers" (Tun-Lin et al. 1995), in the target location. In a previous model calibration (Focks et al. 1993b), predetermined estimates of water flux and assumptions about the degree of shade and water draw-down rates in breeding containers were made using cited literature, unpublished reports and unpublished data, and then iteratively adjusted larval food inputs until pupal crop matched field observation. Although such model fitting to field observation serves to calibrate the model, true field validation is required for the model to then be used for estimating population size. To the best of our knowledge, there are no published reports of true field validation based only on container surveys to test the accuracy of calibrated CIMSiM models for estimating population size. Previous use of CIMSiM in northern Queensland (Maguire et al. 1999) has not included calibration or validation of any kind.

In this study, we describe a novel variation to the standard calibration method of the CIMSiM platform for *Ae. aegypti* productivity by deliberately placing "sentinel" key containers in the field in far north Queensland (Qld), Australia. We describe the measurement of water flux and pupal productivity in the containers over time, permitting the adjustment of CIMSiM so that simulated pupal productivity matched that of sentinel key containers. We then tested this calibrated model in blind field validations. Our overall aim was to create a calibrated and field-validated model that could then be used to describe accurately and rapidly the size (i.e., pupal crop per ha.) of *Ae. aegypti* populations from simple container surveys that did not require laborious immature census.

Materials and Methods

Study Area. Cairns in the wet tropics of far north Qld has a human population of ≈125,000, and it is subject to dengue outbreaks almost annually (Ritchie et al. 2002, Hanna et al. 2006). Annual rainfall in the wet tropics ranges from 2,000 to 4,500 mm, with average daily min.-max temperatures of 19–29°C (Australian Bureau of Meteorology; www.bom.gov.au). The primary DENV vector in Cairns is *Ae. aegypti*, which is the most prevalent container-breeding mosquito in the region. *Aedes albopictus* (Skuse), another prominent DENV vector, is present in the Torres Strait north of Qld (Ritchie et al. 2006) but is absent from Cairns (S.A.R. and S.A.L., unpublished observations).

Selection of Sentinel Key Container Types. To determine which container categories to use as sentinel key calibrating containers, recent surveys of *Ae. aegypti*-positive containers in the Cairns area (S.A.R. and S.A.L., unpublished observations) were studied. Despite the presence of *Ae. aegypti* in several dozen container types, it was apparent that plastic buckets, pot plant bases, used car tires, and indentations in tarpaulins (including plastic sheeting) accounted for ≈60% of containers and 73% of *Ae. aegypti* pupae (P.H.J., unpublished data). Furthermore, the diverse size and shape of these containers represents a diversity of water flux dynamics, and their portability meant they were convenient to use.

Deployment and Observations of Sentinel Key Containers. Replicates of each container type (Table 1) were deployed at seven locations in the greater Cairns area, separated by up to 30 km. The containers were placed outdoors on residential properties in well shaded areas typical of such containers in Cairns: pot

Table 1. Sentinel key container types used for field observations of water flux and *Ae. aegypti* pupal productivity in Cairns, Australia

Container	Mean dimensions	Mean capacity (ml)	*n*
Plastic buckets	18 cm diam., 17.4 cm ht	4,871	7
Pot plant bases	6.13 by 2.04 by 26.0 cm	613	7
Tires (car)	35.3 cm i.d., 11.0 cm inner width	5,343	7
Tarpaulins	15.5 by 22.6 by 4.2 cm (folds collect water)[a]	520	14[b]

[a] Tarpaulin depressions were created by fitting tarpaulins snugly into a 16- by 23- by 4-cm plastic take-out container and securing with bulldog clips.

[b] Two folds per tarpaulin.

plant bases under cover on verandahs, plastic buckets under trees or shrubs, tarpaulins in a shaded part of the yard adjacent to the house, and tires resting at a 20–30° angle against a fence. Containers were placed in the field in December 2006 (early wet season) to be exposed to natural outdoor conditions for 1 mo before observations. This allowed natural recruitment of mosquitoes into containers and ensured that any residual compounds in the containers that may deter mosquito oviposition (such as plasticizers in buckets) were minimized. Formal observations commenced on 9 January 2007, and they were performed twice weekly until 13 March 2007, yielding 19 observations per container over 72 d.

For each observation, water volume was determined by scooping or tipping water into a graduated measuring jug. Pupae were removed and counted by either direct pipetting or gently sieving the water through fine mesh. Fresh pupal skins from recently emerged adults also were collected in the field. After measurement, mosquito larvae, water, and any solid organic material were gently returned to the sentinel key container. Pupae were returned live to the laboratory for emergence to determine the proportion of mosquitoes that were *Ae. aegypti.* This proportion was used to calculate the number of *Ae. aegypti* from field-collected pupal skins and the total number of pupae present on that day.

Meteorological Data. Daily observations of rainfall, temperature, and relative humidity for Cairns Airport were obtained from the Australian Bureau of Meteorology (www.bom.gov.au). The saturation deficit (mb) was calculated for each day. Despite the distance between Cairns Airport and some of the sentinel key containers (up to ≈30 km), having replicate containers in a variety of locations would enable calculation of average water flux in the containers for the Cairns region generally, accounting for local geographic variation in rainfall. In addition, rainfall at three sentinel key container locations was recorded in household gauges to determine the extent of its variability throughout the Cairns region so that the impact of this on water flux in containers could be assessed.

Population Simulation. CIMSiM (MS-DOS version 1.4, USDA, Gainesville, FL) was used to estimate container filling and populations of *Ae. aegypti* in Cairns. Despite its creation some years ago (1995), the algorithms contained within were still considered valid and no later version was thought to be superior. *Ae. aegypti* biology parameters devised for CIMSiM (Focks et al. 1993a) were used unaltered, with the exception of the probability of daily adult survivorship, which was adjusted from 0.89 (default value in CIMSiM) to 0.83. This estimate was made in the course of locally conducted mark–release–recapture and cage experiments (P.H.J. and L.P.R., unpublished observations).

All CIMSiM simulations were performed for 2 yr from 2006 to 2007. The former was treated as an equilibration year (as per Focks et al. 1993b) to permit the initialized population to stabilize. Virtual containers in CIMSiM were initialized with eggs on 1 January 2006 as follows: plastic buckets, 20 eggs; pot plant bases, 20 eggs; tarpaulins, 20 eggs; and tires, 50 eggs. The choice of initial egg numbers was based on unpublished data of breeding containers in the field. The precise number introduced was not thought to be critical in terms of the 2-yr time scale of the simulations performed. CIMSiM performs simulations at the per ha scale. For the purposes of calibration, only the 28 sentinel containers were included in the simulation. We calculated that the density of each container type per ha was pot plant bases, 4.17 per ha; plastic buckets, 4.17 per ha; tires, 4.17 per ha; and tarpaulins, 8.33 per ha.

Calibration of Water Flux. A 2-yr simulation in CIMSiM was repeatedly performed with the sentinel key containers. Water shed ratios and sun exposure values in CIMSiM were iteratively adjusted with each simulation so that water levels in sentinel key containers adequately matched simulated containers on the 19 sampling dates in 2007. Pot plant bases are manually filled by residents watering potted plants and largely uninfluenced by rainfall, so they were always filled to a set volume (350 ml) in CIMSiM. Spearman Correlation (STATA 9.0, Stata Corporation, College Station, TX) was used to test the accuracy of simulated water flux. We considered that adequate fit between simulated and real values had been achieved if we could not improve the fit with further iterative adjustments and that there was a statistically significant correlation ($P < 0.05$) and $r > 0.75$.

Calibration of Pupal Productivity. After water flux in sentinel key containers was calibrated, simulations were again performed. Monthly food delivery into each simulated container was iteratively adjusted until mean pupal productivity across the 19 sampling dates adequately matched actual pupal numbers. This iterative adjustment of average food delivery for CIMSiM calibration is the recommended method for fitting the model to different environments (Focks et al. 1993b). Student's *t*-test (STATA 9.0) was used to compare iteratively matched pupal productivity (pupae per ha) in sentinel key containers with simulated containers on the 19 sampling dates to test the accuracy of simulated pupal productivity. We considered that adequate fit between simulated and real values had been achieved if we could not improve the fit with further iterative adjustments, and that there was no significant difference according to *t*-test results.

Field Validation of CIMSiM Pupal Crop Estimates. The newly calibrated CIMSiM model was tested for the ability to accurately estimate per ha pupal crop in 175 premises (house or unit block with yard) in the Cairns suburb of Parramatta Park in 2007. Surveys of potential *Ae. aegypti* breeding containers were conducted in the wet season (157 premises, 29 January–7 February) and the dry season (149 premises, 20–22 August). Pupae were counted from each container and emerging adults identified in the laboratory. Complete details of *Ae. aegypti* container surveys will be reported elsewhere.

Per hectare densities of the four container types (plastic buckets, pot plant bases, tires, and tarpaulins) were calculated from the wet season breeding con-

Table 2. CIMSiM descriptive values for calibrated containers

Parameter	Plastic buckets	Pot plant bases	Tarpaulins	Tires
Sun exposure	0.2	0	0.8	0.3
Container cover	0	1	0	1
Water shed ratio	1.2	0	0.6	0.2
Draw down (liters)	0	0.3	0	0
Initial food (mg)	70	100	450	500
Mean food per d (mg)	30	10	175	100

tainer survey. These densities were then entered into CIMSiM, and 10 replicate simulations for 2006–2007 were performed. Although average daily food delivery into containers was determined from the earlier calibration (see above), the daily delivery rate was then set to "random" in CIMSiM for the validation. This feature allows the simulations to behave stochastically, with each simulation providing a different pupal value. Random food delivery in CIMSiM is facilitated by the generation of random numbers from zero—twice the daily stated food delivery value (Table 2). Random amounts of food in this range are delivered each day with the result being that the average daily food is delivered overall. Mean pupal crop with 95% confidence intervals were calculated for 10-d intervals coinciding with the two container survey periods: day 29–38 (29 January–7 February) and day 228–237 (20–22 August). Despite the 3-d duration of the August survey, we chose a 10-d interval for comparison in CIMSiM for consistency with our wet season comparison. Validation comparisons were only made for the four previously calibrated container types described above, which formed a large proportion of the containers in the surveys. Other *Ae. aegypti*-positive container types in Parramatta Park were not considered here.

Results

Calibration of Water Flux in Sentinel Key Containers. Container characteristics were iteratively adjusted (Table 2) so that water flux was adequately simulated in CIMSiM and significantly correlated with that of actual sentinel key containers in Cairns (Fig. 2). On most sampling days, the simulated water volume was within 95% confidence intervals of mean volume in sentinel key containers, demonstrating that meteorological data collected at a single point can be used to drive reasonably accurate water flux in simulated containers. However, a 1-wk time lag was observed with the simulated filling of tires (Fig. 2), whereas tarpaulins filled to a greater volume than the actual sentinel key containers, some of which lost water through cracks that developed. These errors could not be rectified by iterative model adjustment.

Household rain gauges at three of the seven sentinel key container locations revealed some local rainfall variation: site 1, 1,104 mm; site 2, 1,136 mm; and site 3, 824 mm. Total rain at Cairns Airport (used to drive CIMSiM simulations) was 874 mm. Daily rainfall readings at all three locations correlated strongly with readings at Cairns Airport ($r = 0.79–0.94, P < 0.0001$).

Calibration of Pupal Productivity. Iterative adjustments of food delivery rates (Focks et al. 1993b) al-

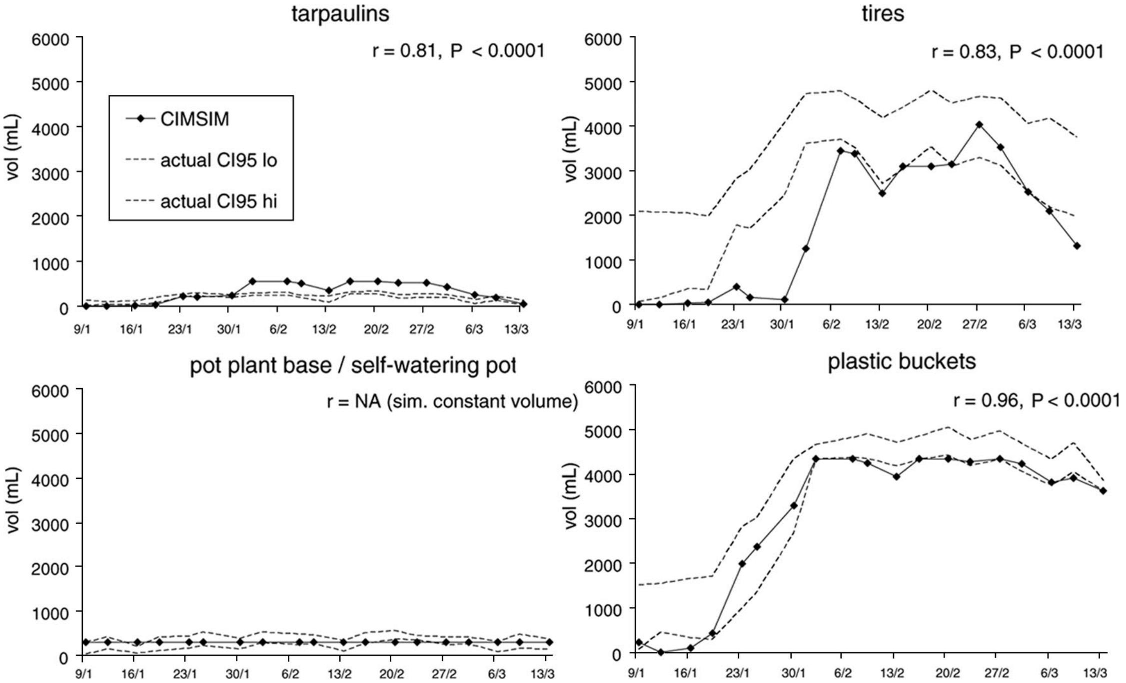

Fig. 2. Calibration of water flux in sentinel key containers in greater Cairns, Australia, on 19 sampling dates from 9 January–13 March 2007, with Spearman correlation analysis.

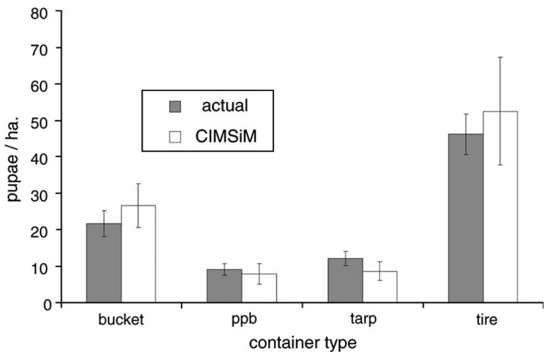

Fig. 3. Calibration of pupal productivity in simulated and four sentinel key containers in greater Cairns, Australia, on 19 sampling dates from 9 January–13 March 2007.

lowed the calibration of pupal productivity in simulated containers so that it adequately matched actual pupal numbers in sentinel key containers (Fig. 3). No significant differences between actual mean daily pupal productivity and CIMSiM productivity were observed: plastic buckets, $t = 0.7059$, $P = 0.49$; pot plant bases, $t = 0.3943$, $P = 0.70$; tarpaulins, $t = 1.0995$, $P = 0.28$; and tires $t = 0.3997$, $P = 0.69$.

Model Validation by Comparison of Simulated and Actual Pupal Productivity. Actual pupae per ha. in Parramatta Park on two 10-d periods in 2007 fell within 95% confidence intervals of simulated pupal crop estimates made by 10 replicate simulations in CIMSiM (Table 3). Our wet season CIMSiM estimate was slightly lower (by ≈4 pupae per ha) than the actual value, whereas our dry season estimate was slightly higher than actual (by ≈3 pupae per ha). Nonetheless, by generating estimated confidence intervals in CIMSiM that encompassed the actual pupae per ha value, a successful validation of the model was achieved.

Despite this validation, closer examination of pupal productivity in each container type revealed that although we successfully estimated overall pupal productivity across all container types, the ability of CIMSiM to estimate productivity in each container was inconsistent (Table 3). In the wet season we overestimated production in tires and underestimated in tarpaulins and plastic buckets. In the dry season, CIMSiM predicted pupal productivity only in pot plant bases during the sampling period. However, it is worth noting that in CIMSiM pupae were produced in the other container types on other dates in the dry season.

Table 3. Comparison of actual and simulated pupal productivity of four container types in suburban Cairns in the wet and dry seasons of 2007

Container	Pupal productivity	
	Actual pupae per ha	CIMSiM estimate (CI95)
	Wet season (29 Jan.–7 Feb.)	
Plastic buckets	28.2	19.6 (15.0–24.1)
Pot plant bases	22.6	26.4 (18.0–34.8)
Tires (car)	4.7	16.0 (14.2–17.7)
Tarpaulins	14.4	4.1 (3.5–4.7)
Total	70.0	66.1 (56.0–76.2)
	Dry season (20–22 Aug.)	
Plastic buckets	1.9	0
Pot plant bases	0.3	9.6 (2.6–16.6)
Tires (car)	0	0
Tarpaulins	4.5	0
Total	6.7	9.6 (2.6–16.6)

Discussion

Using only container survey data describing the density per ha of *Ae. aegypti*-positive containers and readily available meteorological data, accurate estimates of pupal crop in suburban Cairns were made for two periods in 2007 by using a newly calibrated CIMSiM model. The accuracy of these estimates was validated by comparison with the actual pupal counts recorded during the same container survey. The use of CIMSiM with the described calibration/validation process represents a novel and extremely rapid method for population size estimation. A container survey of a typical north Queensland suburb (100–200 premises) in which containers are scored as either positive or negative for *Ae. aegypti* immatures, but the number of *Ae. aegypti* larvae/pupae is not recorded, takes two to three workers 1–2 d. Using a calibrated CIMSiM model, these basic container survey data and recent meteorological data, accurate estimates of standing pupal crop could be made within a day. Thus, an estimate of population size for a suburb could be made in as little as 2–3 d. This provides a relatively rapid estimate; potentially timely enough for the planned release of transgenic or *Wolbachia*-infected mosquitoes. In contrast, a complete survey of *Ae. aegypti* immatures, in which all larvae and pupae in a suburb are identified and counted could take 7 d or longer, depending on *Ae. aegypti* density. Genetic analyses to determine the effective breeding size of the population also would take presumably longer again.

To the best of our knowledge, the study described here is the first to use CIMSiM for population size estimates. This is also the first true validation of CIMSiM testing its ability to estimate population size. To enable this novel application, we had to conduct a calibration study using sentinel key containers in Cairns. Our virtual containers in CIMSiM filled and emptied as sentinel key containers did (water flux calibration), and pupal productivity was matched in the model and in the field (pupal productivity calibration). This two-step calibration resulted in a model that could be used to estimate standing pupal crop.

CIMSiM has been used to describe seasonal changes in *Ae. aegypti* populations in north Queensland, in the city of Townsville (Maguire et al. 1999), a similar size to Cairns. Although container survey was used to parameterize the CIMSiM model to perform simulations, there were no reported calibration or validation procedures performed, meaning that the estimates of *Ae. aegypti* per ha reported therein were purely speculative.

Focks et al. (1993b) established that when pupal crop estimates in CIMSiM match those in the field, then other simulated population parameters also match. Thus, by successfully estimating pupal crop in the field by using CIMSiM, we may take other aspects of model outputs, such as standing egg, larvae and adult crops, and consider them to also be well estimated. Further validation may be required to confirm this. In this way, the CIMSiM *Ae. aegypti* model is a powerful tool for rapidly estimating various population parameters in the field. Such information will be vital for developing release strategies for modifying mosquito populations and measuring dengue transmission risk. This said, the true utility of simulation modeling for population size estimation will not be fully understood until the realities of rearing large numbers of transgenic or *Wolbachia*-infected mosquitoes for field release are clear. For example, population estimates may be required weeks or months in advance of planned release dates. The ability of CIMSiM to accurately forecast populations in advance is untested.

Despite the initial success reported here, there are a number of limitations in using simulation models for population size estimation. Foremost, our calibrated model was only validated for four container types (plastic buckets, pot plant bases, tarpaulins, and tires). Significant pupal productivity in other containers types cannot currently be estimated and requires a calibration and validation procedure as performed here. Indeed, our four calibrated container types accounted for 73% of the pupae (659/901) in the wet season pupal survey of Parramatta Park (P.H.J., unpublished data). Palm fronds and large plastic containers (e.g., rubbish bins) were also significant producers of pupae that were not simulated here. Future calibration of water flux and pupal productivity of other major *Ae. aegypti*-producing containers is required to strengthen the CIMSiM model.

As with most attempts to model natural populations, several assumptions are inherent. The probability of daily adult survivorship is held constant in the model and is likely to be a source of error. In reality, this is likely to fluctuate with changing weather conditions and may be age-dependent (Harrington et al. 2001). Furthermore, there are few published estimates of *Ae. aegypti* adult daily survivorship available, and none thus far for Queensland (we used unpublished estimates). More field-based estimates of this parameter and how it changes with time will be valuable for modeling purposes. A further assumption we used is that the density of different container types is constant year-round. Further data collection is required so that this can be modeled in CIMSiM.

Meteorological data from a single source, collected over a minimum 2-yr period, is required to drive CIMSiM. However, rainfall is geographically stochastic, meaning that data collected as close to the area for which a simulation is performed is most desirable. Our brief study of rainfall here revealed some local variation in rainfall totals, but strong correlation with that recorded at the nearest meteorological station (Cairns Airport). For the purposes of our calibration, there was sufficient similarity in rainfall between sentinel key container sites and the meteorological station. However, we recommend that for future simulations in new locations, the extent of local rainfall variation be examined.

The success in validating CIMSiM for north Qld *Ae. aegypti* was in estimating overall pupal productivity in four container types. We are hopeful that the calibration data and the approach adopted here may be applicable to other regions and to alternative *Ae. aegypti* models developed by other workers (e.g., Skeeter Buster, K. Magori, M. Legros, Alun Lloyd, and F. Gould, personal communication). As the calibration parameters are only valid for north Qld, we recommend that new calibrations are conducted for each new region in which CIMSiM is to be applied. The number of sentinel containers used, and the range over which they are deployed, will depend upon the extent of heterogeneity in *Ae. aegypti* breeding ecology in the study area. A large, heterogeneous area would presumably require a larger number of sentinel containers deployed over a larger area compared with a homogeneous study area. For our calibration, 28 sentinel containers (seven replicates of each of the four categories) spread uniformly over ≈50 km^2 sufficed to provide calibrated parameters that could be field validated. Experienced local field officers would need to decide how best to conduct the calibration in their own study area.

Our ability to estimate pupal numbers in each container type on specific dates was inconsistent. In reality, the stochasticity of the field environment can never be wholly simulated. For this reason, the modeling approach described here can never wholly replace *Ae. aegypti* immature survey. Our best efforts with CIMSiM will only ever provide us with estimates of overall pupal productivity, with some associated error. Understanding the capabilities and limitations of the modeling approach to population size estimation will be crucial in its successful application.

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