Novel Estimates of *Aedes aegypti* (Diptera: Culicidae) Population Size and Adult Survival Based on *Wolbachia* Releases

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ABSTRACT The size of *Aedes aegypti* (L.) mosquito populations and adult survival rates have proven difficult to estimate because of a lack of consistent quantitative measures to equate sampling methods, such as adult trapping, to actual population size. However, such estimates are critical for devising control methods and for modeling the transmission of dengue and other infectious agents carried by this species. Here we take advantage of recent releases of *Wolbachia*-infected *Ae. aegypti* coupled with the results of ongoing monitoring to estimate the size of adult *Ae. aegypti* populations around Cairns in far north Queensland, Australia. Based on the association between released adults infected with *Wolbachia* and data from Biogents Sentinel traps, we show that data from two locations are consistent with population estimates of ≈5–10 females per house and daily survival rates of 0.7–0.9 for the released *Wolbachia*-infected females. Moreover, we estimate that networks of Biogents Sentinel traps at a density of one per 15 houses capture around 5–10% of the adult population per week, and provide a rapid estimate of the absolute population size of *Ae. aegypti.* These data are discussed with respect to release rates and monitoring in future *Wolbachia* releases and also the levels of suppression required to reduce dengue transmission.

KEY WORDS *Aedes aegypti*, *Wolbachia*, dengue, BGS trap, surveillance

An understanding of the size of populations of *Aedes aegypti* (L.) is critical for many control operations (Morrison et al. 2008). It most cases, the absolute population in numbers per unit area is unknown, so relative population size is used. This may consist of relative changes in trap collections, such as fluctuations in weekly collections over time, or comparison of trap collections between sites or even different treatment regimes. Such trap methods usually have inherent biases, and may selectively collect or sample specific physiological stages or sexes. For example, ovitraps selectively sample gravid females, and trap efficacy can be impacted by the presence of competing natural containers, especially large containers already positive for *Ae. aegypti* (Harrington et al. 2008, Wong et al. 2011). Biogents Sentinel (BGS) traps (Krockel et al. 2006, Williams et al. 2006) under-sample teneral and bloodfed females (Ball and Ritchie 2010a), and trap efficiency can be impacted by competing dark objects (Ball and Ritchie 2010b). However, sampling a specific stage or sex may not be inherently biased provided the frequency of the stage sampled is relatively consistent among aquatic and adult stages. Container surveys targeting the larval and pupal stages have also been used to estimate relative population size, and (in the case of pupae) absolute population size (Focks and Chadee 1997, Focks et al. 2000, Morrison et al. 2008, Williams et al. in press). However, container and pupal surveys are laborious, and often miss so-called cryptic breeding sites that are hard to locate and difficult to access. This includes subterranean containers such as sump pits (Montgomery et al. 2004) and telecommunication pits (Kay et al. 2000), and elevated sites such as roof gutters (Montgomery and Ritchie 2002) and rainwater tanks (Hanna et al. 1998), all of which can be highly productive key containers within their locale.

Mark release recapture (MRR) studies have been used to estimate the absolute population size of several insects (Southwood 1978). For *Ae. aegypti,* this usually consists of releasing cohorts of adult *Ae. aegypti* that have been marked with fluorescent dust or paints (Sheppard et al. 1969, Trpis et al. 1995, Edman et al. 1998, Harrington et al. 2005). The released mosquitoes then disperse and mix with the natural population. Both populations are then sampled, and the ratio of marked to unmarked mosquitoes used to estimate the size of the natural population (the Lincoln Index, or modifications thereof) (Sheppard et al. 1969, Southwood 1978, Trpis et al. 1995).

Here we use the release of thousands of *Ae. aegypti* infected with *Wolbachia* (*w*Mel strain) into uninfected *Ae. aegypti* populations to estimate the size of

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the wild *Ae. aegypti* adult population. Bellini et al. (2010) previously used *Wolbachia* in a MRR to estimate dispersal and survival in population of *Aedes albopictus* (Skuse) in Italy. In their case, *Ae. albopictus* were naturally infected with *Wolbachia,* and *Ae. albopictus* free of *Wolbachia* served as the marked cohort. This cohort was produced by rearing larvae in the presence of antibiotics to remove the *Wolbachia* infection. In our situation, isolated urban areas consisting of 600–700 premises were subject to weekly releases of known numbers of *Wolbachia*-infected *Ae. aegypti* (Hoffmann et al. 2011). The communities were geographically confined by water (Coral Sea), mangroves or sugar cane fields, thus migration of *Ae. aegypti* into and out of the release area would have been minimal, as *Ae. aegypti* is largely restricted to urban areas.

The initial release cohorts were used to estimate population size and daily survival rates. We did not analyze captured *Ae. aegypti* for the presence of *Wolbachia,* but rather used the relative increase in BGS captures of female *Ae. aegypti* after releases to estimate the relative sizes of the released and wild female *Ae. aegypti* populations. We also examined the accuracy and utility of BGS collections to estimate the size of the wild population and the survival of released *Wolbachia*-infected *Ae. aegypti* using regression analyses.

Materials and Methods

Release and Monitoring Areas. Estimates were obtained from *Wolbachia* releases at two locations. Starting on 4 January 2011, once a week cups of 2–4 d old adult male and female (1:1 sex ratio, with 10 released/house) *Ae. aegypti* infected with the *w*Mel strain of *Wolbachia* (Walker et al. 2011) were release in Gordonvale (23 km south east of Cairns) and in Yorkeys Knob (17 km northwest of Cairns) north Queensland, Australia (Hoffmann et al. 2011). For this analysis, mosquitoes were assumed to have been released evenly throughout the release area (they took place at every fourth house, with each release house receiving the ca. same number of mosquitoes (≈40 females; Hoffmann et al. 2011). Moreover, each release area was assumed to be isolated and the extant *Ae. aegypti* population considered closed. In support of this assumption, there was only a very low level of movement of infected mosquitoes outside the release area (Hoffmann et al. 2011).

Mosquito numbers were monitored with BGS traps set outdoors in covered areas such as under highset 'Queenslander' houses (an elevated pole house) and in carports and laundry areas adjacent to a house. Traps (12–20 traps per run/wk/release area) were run continuously and data expressed as mean number of female *Ae. aegypti*/trap day. The BGS catches were collected every Thursday and releases were carried out every Wednesday. Thus, there was a 1-d period when BGSs could have collected mosquitoes from a new release. Captures of *Ae. aegypti* from 12 BGS traps placed in the Parramatta Park suburb of Cairns (2010–2011) that was free of released mosquitoes served as a control. Male *Ae. aegypti* captures from all BGS traps tended to be low and variable, and were therefore not included in the analyses. All estimates of population size were based on changes in BGS counts, not on *Wolbachia* numbers (i.e., *Wolbachia* was not directly used as a marker to estimate population size), although all releases were confirmed as *Wolbachia* infected mosquitoes by a polymerase chain reaction (PCR) assay (Lee et al. 2012).

Estimation of the Population of Wild *Ae. aegypti* From Relative Changes in BGS Trap Collections. We estimated the population size of wild female *Ae. aegypti* based on the relative increase of female *Ae. aegypti* captures in BGS traps in response to the release of *Wolbachia*-infected females (Fig. 1; Table 1). Collections made 2 wk or later after release are confounded by emergence of the F1 generation from the initial release cohort, and therefore were not used. We tested if BGS captures of female *Ae. aegypti* increased significantly after releases by comparing the mean number of female *Ae. aegypti*/BGS trap day for the 2 wk before and after release using *t*-tests on log $(x + 1)$ transformed counts. We also assumed equal capture rates for *Wolbachia*-infected versus wild female *Ae. aegypti* across all ages. While *Wolbachia* infection can impact adult survival (e.g., the *w*Melpop strain; McMeniman et al. 2009), the *w*Mel strain we used decreased adult female survival by ≈10% or less (Walker et al. 2011).

To estimate the wild female population, we computed the difference between BGS catches one and 2 wk before and after the mosquito release, and used the proportional increase as an estimate of the relative size of the released cohort to wild *Ae. aegypti* (Table 1). We accounted for mortality of released mosquitoes by computing the expected number of released females per premise per day for an assumed daily survival of 0.7, 0.8, and 0.9 in an Excel spread sheet to produce a daily average of the expected number of released females/premise (Table 2). The expected number of released females per premise during the BGS capture period was then multiplied by the estimated ratio of wild: release mosquitoes to provide an estimate of the number of uninfected females present in the population over the trap collection period (Table 1). We assume that the released infected mosquitoes and uninfected mosquitoes had the same daily survival rates. CIs of estimated wild female *Ae. aegypti* were calculated by 1) calculating the CI of mean BGS captures one and 2 wk before release, 2) dividing the CI by the respective mean to obtain the proportion of the mean for each CI, and 3) multiplying the estimated wild population (calculated from Table 1) by this proportion to estimate the CI for the estimated wild population/premise. This value was then subtracted and added to the estimated wild population to obtain the respective lower and upper CI reported in Table 3.

Estimation of Wild Population of *Ae. aegypti* From Regression of BGS Trap Collections. We obtained a second estimate of population size as well as an estimate of the daily survival rate by using a regression approach incorporating BGS counts from the entire release period as well as the period before release and

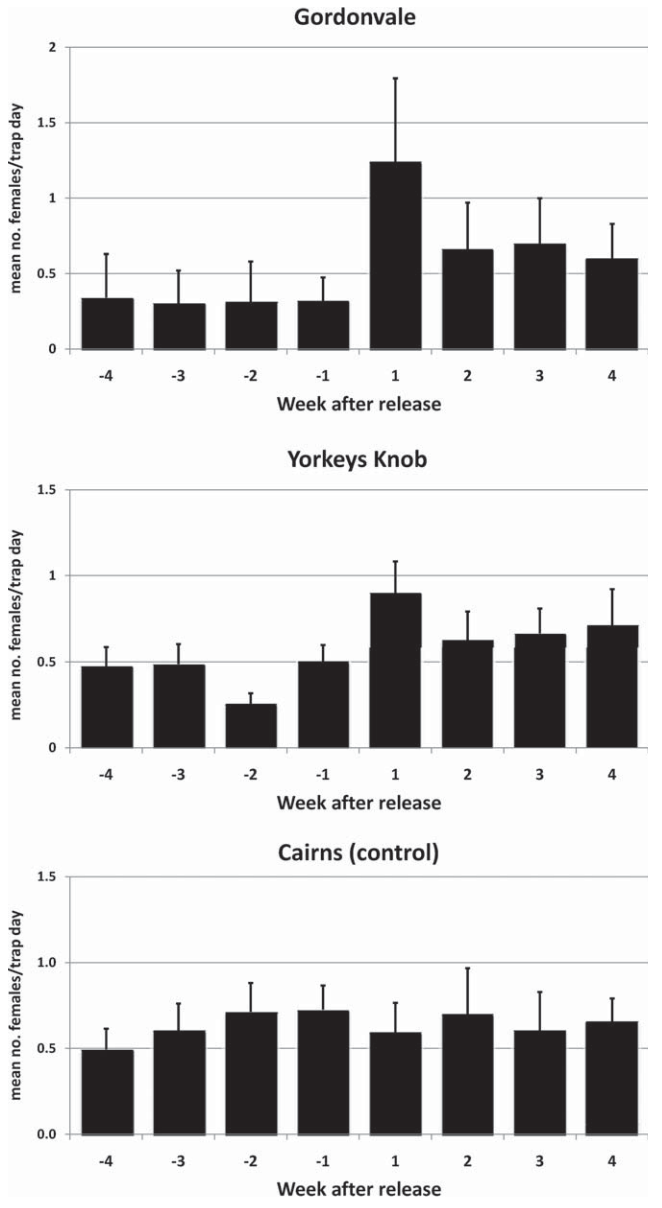

Fig. 1. Mean (+SEM) captures of female *Ae. aegypti* per BGS trap day at the two release sites (top: Gordonvale; middle: Yorkeys Knob; bottom: Cairns control) for the 4 wk before and after release. Note the relatively consistent collections before release followed by a significant increase after release. Population estimates were made by comparing relative change in mean captures 1–2 wk after release to those 1–2 wk before release (Table 1). Mean BGS collections for 2-wk period before and after release were significantly different (*t*-test; $P < 0.05$) except for at the Cairns (Parramatta Park) control site.

after the release. Because releases took place over several weeks, we initially tested if there was a consistent change in BGS catch numbers in the control (Parramatta Park) area across the 10-wk release period as well as the 3 wk before release and 4 wk after releases stopped. Based on the association between numbers from Gordonvale and Parramatta Park (see Results below), numbers were corrected for the decrease in Parramatta Park in the 17 wk during and around the release period using linear regression, but Yorkeys Knob data were not corrected.

By varying daily survival (DS) rates by 0.05 in the range 0.50–0.95, we estimated survival rates that provided the best fit (by least squares) for the relationship between BGS counts (or residuals when these had been adjusted for control area scores) and the expected size of the adult release population. We used BGS counts from the 10-wk period during release and the ensuing 4-wk period when a substantial number of released adults were expected to remain in the population. An assumption in this analysis is that the total numbers of mosquitoes emerging from natural breed-

Table 1. Calculations for estimating the number of wild female *Ae. aegypti*/premise from releases of *Wolbachia*-infected *Ae. aegypti* using BGS capture data

Variable	Sample calculation
1. Mean no. of wild female *Ae. aegypti* per BGS trap (either 1 or 2 wk before release; see Fig. 1)	1.0
2. Mean no. of female *Ae. aegypti* per BGS trap after (either 1 or 2 wk) release of *Wolbachia*-infected mosquitoes	3.0
3. Increase in BGS collections of female *Ae. aegypti* due to due to released mosquitoes	3.0–1.0 = 2.0
4. Ratio of wild to released mosquitoes in BGS collections	1.0/2.0 = 0.5
5. Estimated no. of wild mosquitoes per house = no. of released mosquitoes/house X ratio of wild to released mosquitoes[a]	10 by 0.5 = 5

Wild female *Ae. aegypti* populations were estimated from no. of released mosquitoes and relative change in BGS counts. CIs are based on CIs from captures made either 1 or 2 wk(s) before release.

[a] This does not take into account loss of released mosquitoes by death or immigration. For calculations of released mosquitoes incorporating mortality see Table 2.

ing sites (including any infected mosquitoes successfully bred in the field) do not change during the 14-wk period. This is supported by a study by (Williams et al. in press) that found that *Ae. aegypti* pupal production from key containers was relatively consistent during the wet season in Cairns. We also included the 3-wk period before release in estimating survival (although using only data from the release and postrelease periods provided similar results). We initially assumed a linear relationship between BGS counts and release numbers but also tested for a nonlinear relationship by adding a polynomial term in case BGS traps were less efficient at very high release rates. Based on the fitted estimates of DS and regression lines, we then computed the predicted size of the populations before *Wolbachia* was introduced.

Estimation of Efficiency of BGS Traps. The efficiency of the BGS trap (field sampling rate of Johnson et al. 2012) to collect released female *Ae. aegypti* was estimated using the expected number of released mosquitoes per premise at Gordonvale and Yorkeys Knob, with the increase in mean number of female *Ae. aegypti* per BGS trap day after release attributed to the release cohort (Fig. 1). This value was divided by the expected number of released mosquitoes per premise for daily survival of 0.7, 0.8, and 0.9 to estimate the BGS trap efficiency. This was done for BGS collections made 1 and 2 wk after release.

Results

Estimation of Wild Population of *Ae. aegypti* From Relative Changes in BGS Trap Collections. The relative increase in populations after the initial release period can be seen graphically by comparing the mean number of females collected by BGS traps set in the release areas and the control area (Fig. 1). The BGS sample increased by 1.5–2× for the initial 2 wk of releases, and was significantly higher (*t*-test; $P < 0.05$) for both release sites. Concurrent collections in the Cairns (Parramatta Park) control areas in 2011 did not change significantly ($t = 0.204$; $P = 0.840$) for 2 wk after releases.

We estimated the expected size of the released infected mosquito populations through an exponential model with different levels of daily survival in the range 0.7- 0.9 (Table 3). The expected number of *Wolbachia* infected females/house ranged from ≈4.5–5 for DS of 0.7 to ≈7.5–10 for DS of 0.9. Expanding the BGS data used in calculations from 1 to 2 wk before and after release did not change the estimated populations much, but did tighten the CI because of the larger total number of females collected (Table 3). Based on these figures, the estimated uninfected wild population ranged from 4.5 to 6 per premise for a daily survival rate of 0.7, to 8.5–10 per premise for a daily survival rate of 0.9. Doubling of the BGS sampling

Table 2. Estimation of expected no. of released *Wolbachia*-infected female *Ae. aegypti* per premise for an assumed daily survival rate of 0.7, 0.8, and 0.9 for releases at Yorkeys Knob on 4 Jan. 2011

Date	BGS trap day	Females released/premise	Females/premise for specified daily survival		
			0.7	0.8	0.9
4 January 2011	Not set	8.6	8.6	8.6	8.6
5 January 2011	Not set		6.0	6.9	7.7
6 January 2011	1 trap set		4.2	5.5	7.0
7 January 2011	2		3.0	4.4	6.3
8 January 2011	3		2.1	3.5	5.6
9 January 2011	4		1.4	2.8	5.1
10 January 2011	5		1.0	2.3	4.6
11 January 2011	6	8.8	9.5	10.6	12.9
12 January 2011	7 trap picked up		6.7	8.5	11.6
	Total females for trap week (6–12 January 2011)		27.9	37.6	53.1
	Females/premise/d		4.0	5.4	7.6

Estimated populations used in calculation of total females/BGS trap week are shaded grey.

Table 3. Estimated no. of wild female *Ae. aegypti*/premise based on three levels of DS

Locale/parameter	1 wk before/after initial release			2 wk before/after initial release		
	DS = 0.7	DS = 0.8	DS = 0.9	DS = 0.7	DS = 0.8	DS = 0.9
Gordonvale (668 premises)						
Female *Ae. aegypti* released in weekly cohort (no./house)[a]		7,203 (10.8)			6,862 (10.3)	
Mean BGS collection before, after release[a]		0.71, 1.40			0.65, 1.34	
Increase in mean BGS collection because of release cohort, ratio wild to released[a]		0.69, 1.03			0.69, 0.94	
Expected no./premise for different DS	4.71	6.09	8.04	4.79	6.58	9.77
Wild female *Ae. aegypti*/premise	4.85	6.26	8.27	4.50	6.20	9.18
95% CI wild females	2.42–7.27	3.13–9.39	4.13–12.40	2.74–6.25	3.79–8.61	5.61–12.75
Yorkeys Knob (614 premises)						
Female *Ae. aegypti* released in weekly cohort (no./house)[a]		5,286 (8.7)			5,413 (8.8)	
Mean BGS collection before, after release[a]		0.50, 0.90			0.38, 0.76	
Increase in mean BGS collection because of release cohort, ratio wild to released[a]		0.40, 1.25			0.38, 1.00	
Expected no./premise for different DS	4.53	5.82	7.76	4.89	6.57	9.67
Wild female *Ae. aegypti*/premise	5.66	7.27	9.71	4.50	6.20	9.18
95% CI	3.40–7.93	4.36–10.18	4.95–14.46	3.32–6.45	4.47–8.67	6.57–13.15

Wild female *Ae. aegypti* populations were estimated from no. of released mosquitoes and relative change in BGS counts. CIs are based on CIs for BGS counts from 1 or 2 wk(s) before/after release.
[a] Daily survival rates not applicable.

period did not change estimates much although CIs were smaller because of larger number of trappings.

Estimation of Wild Population of *Ae. aegypti* From Regression of BGS Trap Collections. For the Parramatta Park collections outside the release area, there was a significant ($P < 0.05$) decrease in BGS counts in the 17 wk period during, before and after the release period, although this trend was weak (linear regression, $R^2 = 0.22$). In the 12-wk period before releases started, weekly BGS counts in Gordonvale were correlated to those from Parramatta Park ($r = 0.54$; $P < 0.01$), but those from Yorkeys Knob were not ($r = 0.06$; $P > 0.05$). Therefore, we corrected the Gordonvale data for the Parramatta Park numbers by linear regression and used residuals, but did not correct the Yorkeys Knob numbers.

For the Gordonvale data set, the (corrected) BGS counts showed a linear relationship with release numbers ($R^2 > 0.7$). For (uncorrected) Yorkeys Knob data, the addition of a polynomial term improved the fit significantly ($P < 0.01$ under a range of survival estimates). BGS trap counts could be related to release numbers in both release sites ($R^2 > 0.7$) under different fitted values for daily survival rates. The survival estimate providing the best fit was lower for Gordonvale than for Yorkeys Knob (Table 4). Population sizes before release were estimated to be similar at two release sites. However, CIs were quite wide particularly for Yorkeys Knob (Table 4) and overlapped with estimates obtained with the other approach (c.f. Table 3).

Estimation of Efficiency of BGS Traps. The estimated efficiency of the BGS to collect released mosquitoes ranged from ≈4–15%, and increased as the assumed DS of released females decreased, resulting in a smaller population of released mosquitoes that was sampled (Table 5). BGS trap efficiency was estimated to be ≈2× higher in Gordonvale than Yorkeys Knob. Increasing the BGS sampling period from 1 to 2 wk either side of release did not change the efficiency estimates much, although the CIs of the estimated wild population were smaller (Table 3).

Table 4. Estimated survival rates and pop sizes of females before release based on regressions of release size against counts from BGS traps

Population	Estimated survival	R^2	Mean BGS count before release	Mean BGS count after release	Estimated pop before release	Estimate per house	CIs (of estimate per house)
Gordonvale	0.75	0.747	0.766	1.109	7,261	5.53	3.00–7.16
Yorkeys Knob	0.90	0.755	0.363	0.781	7,862	5.88	3.36–20.56

Estimates for Gordonvale were based on linear regressions, while those for Yorkeys Knob were based on nonlinear regressions providing the best fit based on specific survival probabilities. CIs were computed from the 95% CIs of the regression lines.

Table 5. BGS trapping efficiency for the two release sites for 1 wk (1:1) and 2 wk (2:2) collections before and after release based on different daily survival (DS) estimates

	DS = 0.7		DS = 0.8		DS = 0.9	
	1:1	2:2	1:1	2:2	1:1	2:2
Gordonvale	14.6%	14.4%	11.3%	10.5%	8.6%	7.1%
Yorkeys Knob	8.8%	7.8%	6.9%	5.8%	5.2%	3.9%

Discussion

The release of mosquito cohorts can be used to estimate populations of wild mosquitoes. This information is useful in several ways. Population estimates are needed to establish thresholds for release numbers to optimize *Wolbachia* integration given that successful invasion by *Wolbachia* will often depend on exceeding unstable equilibrium points (Turelli and Hoffmann 1991). We used our estimates of *Ae. aegypti* populations to estimate the relative survival of released *Wolbachia* infected mosquitoes. These data were also used to determine the relative efficiency of BGS traps to collect female *Ae. aegypti* and to estimate the standing crop of adult *Ae. aegypti.*

We used different methods to measure the relative size of the released mosquito population and estimate the standing wild crop of *Ae. aegypti.* The simplest and most expedient method to measure the relative size of the released population was to compare the relative increase in mean BGS captures of female *Ae. aegypti* in response to released mosquito cohorts. However, DS needs to be considered in converting this estimate to a numerical estimate of population size. Assuming an exponential model, we computed the expected number of females per house for different survival values and with different periods. Expanding the BGS collections from 1 to 2 wk did not change the estimate much, although the larger sample size did decrease the CI (Table 3). We also applied a regression approach linking BGS values to expected population numbers based on the number of released mosquitoes to estimate survival and standing crop.

We estimated population size and trap efficiency under assumed daily survival of 0.7, 0.8, and 0.9. However, which daily survival is most appropriate? The daily survival of female *Ae. aegypti* from MRR experiments ranges from 0.86 to 0.91 in rural Queensland (Muir and Kay 1998) to 0.74–0.84 in Thailand and Puerto Rico (Harrington et al. 2001), while Reiter (2007) argues that rates between 0.9 and 0.95 are warranted. A DS of 0.93 was obtained for Cairns strain of *Ae. aegypti* under semifield conditions (Ritchie et al. 2011). The default DS used in CIMSiM is 0.91 (Focks et al. 1993). Our regression analysis produced best fits for DS ranging from 0.75 to 0.9 (Table 4). Recapture rates of *w*Mel infected female *Ae. aegypti* in sticky ovitraps in Yorkeys Knob and Gordonvale estimate a DS of ≈0.8–0.85 (H.L.Y., unpublished data). Although survival probabilities may depend on *Wolbachia* infections, we suspect that a DS of around 0.8 is likely in many locations. Both methods provided estimated female *Ae. aegypti* population sizes ranging from 4 to 10/premise, consistent with estimates of 8–24 females/premise obtained from pupal surveys, CIMSiM modeling, and BGS collections by Williams et al. (2103) for the Cairns suburb of Parramatta Park in 2008.

Estimating population size using the release of large cohorts of mosquitoes represents an exceptional circumstance. Population estimation generally involves the release of much smaller cohorts of marked individuals that are then recaptured (Southwood et al. 1972). For example, Trpis et al. (1995) released 1,000 each of marked male and female *Ae. aegypti* to estimate the population of *Ae. aegypti* in an African village, obtaining estimates of ≈24–33 females/house. Other population estimation methods include modeling (Focks et al. 1993; Williams et al. 2008, in press), pupal surveys (Focks et al. 1981, Williams et al. in press), and calibrated adult traps (BGS; Johnson et al. 2012). Release of small cohorts is certainly cheaper then the method we used. Marked cohorts also allow you to estimate daily survival and dispersal. However, if large scale releases of mosquitoes for purposes of population modification (e.g., *Wolbachia*) or population suppression (e.g., sterile insect or RIDL; Alphey et al. 2010) are planned, then it is prudent to use this opportunity to estimate population attributes.

Our estimates of field sampling rates of BGS for adult female *Ae. aegypti* (ranging from 5 to 10%) are lower to those obtained from MRR studies conducted within north Queensland houses (Johnson et al. 2012). Importantly, Johnson et al.'s higher recapture rates (20–30%) were made from marked mosquitoes released and trapped within a house. Our estimates incorporate a larger area (i.e., the entire premise property), including the house, carport, laundry area, external buildings, and the yard. Thus, premise-wide BGS capture rates should be lower than recaptures limited to the house only. The capture rates are likely to vary with location and housing type (Johnson et al. 2012) and density. For daily survival rates of 0.8, our estimated BGS field sampling rate varied from 6% at Yorkeys Knob to ≈11% at Gordonvale. The lower sampling rate at Yorkeys Knob may reflect the higher number of apartment blocks with limited green space within the area. Increased densities of buildings and humans at Yorkeys Knob would increase urban features such as houses, outbuildings, carports, and associated furniture that serve as harborage areas for female *Ae. aegypti.* BGS trapping efficiency can decrease with increasing structure that is visually attractive to female *Ae. aegypti* (Ball et al. 2010b). Trapping efficiency also accounts for the seemingly contradictory larger population size at Yorkeys Knob despite higher collections at Gordonvale (Table 1; Fig. 1). Thus, site-specific estimates of trap efficiency should be acquired, although an estimate of 6–15% seems a reasonable approximation.

The field sampling rate or efficiency of the BGS trap to collect adult *Ae. aegypti* provides a valuable tool for use in dengue control programs. Currently, pupal surveys are used to estimate the standing crop of *Ae. aegypti.* As most pupae successfully eclose into adults within 2 d during summer, adult production can be used to estimate total adult population for different daily survival scenarios. These data have also been used to calibrate simulation models of *Ae. aegypti* (e.g., CIMSiM [Focks and Chadee 1997; Williams et al. 2008, in press] and Skeeter Buster [Magori et al. 2009]) to estimate the populations of eggs, larvae, and pupae needed to produce the adult standing crop. However, pupal surveys are laborious, costly, and prone to error because of missed cryptic breeding sites. The surveys of eggs, larvae, and pupae carried out by Southwood et al. (1972) in their life table analysis of *Ae. aegypti* in a Thai wat were even more laborious, and have yet to be repeated elsewhere. Morrison et al. (2008) states that "Development of a cost-effective, field-appropriate method for estimating adult *Ae. aegypti* densities should be a priority," and lists the BGS trap among three most promising methods. BGS traps may represent a rapid, relatively inexpensive way to measure adult populations (Williams et al. 2006, in press; Johnson et al. 2012). One could, by posing the question "what population of eggs and larvae could produce the observed standing crop of adults?," back calibrate from BGS-derived adult standing crop to estimate populations of immatures for simulation modeling. Estimates of the absolute population of adult *Ae. aegypti* would also be useful in identifying dengue transmission risk with BGS sampling programs in areas with active dengue transmission, and in measuring the true impact of interventions on adult *Ae. aegypti.*

Releases of mosquitoes could be managed to improve estimates from BGS trapping. Releases and trap pick-up should be timed to maximize synchrony. In our cases, trapping occurred the day after releases, thus some released mosquitoes would have been captured by traps included in the 'before release' calculation of mean mosquitoes captured by BGS. However, this did not impact collections significantly (Fig. 1), probably because of the short period of overlap (1 d). Trapping and releases on the same day, or at least trap pick up a day before releases, would eliminate collection of recently released mosquitoes. Other sampling methods for adult females, such as sticky ovitraps and aspirator collections, if applied consistently before and during the release period, could also be used to provide estimates of population size using this procedure. The assumption that released mosquitoes have the same likelihood of being caught in BGS traps than naturally reared mosquitoes also needs testing.

In conclusion, we propose a method for estimating populations of mosquitoes during releases of *Wolbachia*-infected *Ae. aegypti.* The method relies upon the fact that the release area is isolated, and that releases are relatively uniform across the area. We obtained consistent estimates of population size with different approaches. Similar estimates could be obtained in other release programs (sterile insect technique, genetically modified mosquitoes) and potentially in releases with other species such as *Ae. albopictus.*

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