**Vector-Borne Diseases, Surveillance, Prevention**

*Aedes aegypti* Population Sampling Using BG-Sentinel Traps in North Queensland Australia: Statistical Considerations for Trap Deployment and Sampling Strategy

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**ABSTRACT**

BG-Sentinel mosquito traps were trialed as a tool for the rapid assessment (24-h collections) and routine monitoring (72-h collections) of adult *Aedes aegypti* L. populations in north Queensland. Analysis of *Ae. aegypti* collections using BG-Sentinels set in suburban Cairns for 24 h permitted the calculation of sample size for a range of precision levels. Clusters of houses with BG-Sentinels operating continuously for 15 d, with collections every 72 h, also permitted required sample size calculation. Evidence of *Ae. aegypti* spatial clustering at the house scale was revealed, with statistically significant effects detected for all collection days. Less variation was detected at each trap location, with only nine of 32 trap locations revealing significant clustering over time. Trap-out effects through continuous BG-Sentinel operation at a fixed location were absent. The findings support fixed position sampling at 72-h intervals for routine monitoring of *Ae. aegypti* populations in Cairns. Despite the relationship between collections of adult vectors and the incidence of disease remaining unknown, BG-Sentinel collections provide an alternative and less labor-intensive abundance measure for assessing risk of dengue virus transmission and success of dengue vector control programs.

**KEY WORDS** *Aedes aegypti*, BG-Sentinel, heterogeneity, spatial, sampling

The container-breeding mosquito *Aedes aegypti* (L.) is the sole vector of dengue viruses in Cairns, north Queensland, Australia, where regular epidemics occur (Ritchie et al. 2002). In response to dengue cases, vector control officers conduct source reduction and deploy lethal ovitraps (Zeichner and Perich 1999) within a 200-m radius of the case house, and interior insecticide spraying within 100 m (Ritchie 2005). This is an effective way to limit further dengue transmission (Ritchie et al. 2002, Ritchie 2005, Russell et al. 2005), but there is a need to develop an improved adult *Ae. aegypti* surveillance program to further enhance dengue management in north Queensland.

Because of the logistical limitations of larval and pupal sampling, our goal is to develop *Ae. aegypti* surveillance based upon adult collection. Although the sampling of *Ae. aegypti* immature stages has been successfully used in Vietnam to identify productive containers and estimate *Ae. aegypti* abundance (Kay et al. 2002), this approach in north Queensland is limited by the cryptic nature and high diversity of breeding containers among houses (Kay et al. 2000, Hanna et al. 2001, Montgomery and Ritchie 2002). Indeed, many hard-to-reach cryptic sites, such as roof gutters and septic tanks, will not be inspected let alone sampled. Furthermore, established larval abundance indices (Breteau, House, and Container indices) have limited use in assessing adult abundance or dengue transmission risk (Focks 2003). More recent analyses have shown that the Breteau index is not strongly related to dengue infection prevalence (Chadee et al. 2005); thus, larval abundance is a poor measure of entomological risk, whereas the number of female *Ae. aegypti* adults per person can be a risk factor for dengue infection (Rodriguez-Figueroa et al. 1995).

Ideally, *Ae. aegypti* adult surveillance programs should include two components. Rapid assessment of abundance to allow for targeting of particular areas for vector control operations and evaluations, and routine collection over several months to provide a measure of population dynamics in relation to dengue virus activity.

The BG-Sentinel mosquito trap (Biogents GmbH, Regensburg, Germany) is highly effective *Ae. aegypti* sampling device, capturing both males and females in similar quantities (Kroeckel et al. 2006, Williams et al. 2006). Abundance and frequency data from investigations in Cairns (Williams et al. 2006) indicated the...
device could be useful for both rapid assessment and routine monitoring of local *Ae. aegypti* populations.

The aim of this study was to first determine required sample sizes (i.e., number of BG-Sentinels) for a range of precision levels when sampling *Ae. aegypti* populations in Cairns. This will provide guidance on designing future *Ae. aegypti* population sampling strategies in the form of determining required sample size for a fixed precision, or conversely to determine the likely level of precision achievable if the number of traps is predetermined by logistical considerations (e.g., trap cost, acceptance by homeowners). An understanding of precision in population sampling is useful for evaluating what difference in relative abundance will be detectable, either between two or more different locations, or at a single location over time. This was done by investigating the relationship between mean and variance values for *Ae. aegypti* collections in BG-Sentinels deployed for 24 h (rapid abundance assessment) and 72 h (routine monitoring).

Second, to determine whether routine monitoring should be conducted at fixed or randomly selected new positions with every deployment, we investigated the extent of spatial and temporal heterogeneity of *Ae. aegypti* adult abundance among houses. Fixed position trapping is commonplace in rural mosquito monitoring programs using New Jersey (e.g., Easton 1987), EVS (e.g., Russell et al. 1991), and CDC traps (e.g., Andreadis et al. 1994). However, for *Ae. aegypti*, a domestic species with generally low density levels (Reiter and Gubler 1997), continuous trapping at a fixed location may cause localized population reduction, thereby affecting data. Alternatively, spatial heterogeneity in abundance among nearby houses may be too great to permit randomized redeployment of traps. We investigated both of these factors using BG-Sentinels in Cairns. We also determined the most appropriate data transformation procedure for non-normally distributed data from BG-Sentinel collections.

Materials and Methods

Required sample size for rapid abundance assessment (24-h collections). BG-Sentinel traps (Biogents GmbH, Regensburg, Germany) were deployed on 10 occasions at noncontiguous houses dispersed over ~2 km² in suburban Cairns. Traps were set outdoors in areas sheltered from wind, direct sunlight, and rain between 1000 and 1400 h, and retrieved 24 h later. Traps were deployed during the wet season, on each of three dates in December 2005 (48 houses with one trap each), February 2006 (26 houses), and four dates in April 2006 (23 houses on three dates, 25 on the fourth date). On each sampling date, the variance (s²) − mean (m) relationship was examined using Taylor’s power law, s² = amᵇ (Taylor 1961), which can be log transformed to the linear equation log₁₀(s²) = log₁₀(a) + b log₁₀(m). Linear regression of log₁₀ s² on log₁₀ m values from each collection day provides values for log₁₀(a) (intercept) and b (slope). The value a is a scaling factor related to sample size, whereas b is a measure of aggregation (Taylor 1961). Values of a and b were then used to calculate the minimum sample size (n, number of BG-Sentinels) required to sample *Ae. aegypti* with a chosen precision, using the expression n = amᵇ * (t/D)², where m is the expected mean number of *Ae. aegypti* per trap per 24 h, t = 1.96, and D is the desired precision level (standard error/mean) (Southwood and Henderson 2000). Calculations were made for precision levels ranging from 0.10 to 0.35, which we considered an amount of variation that would allow biologically significant abundance differences to be apparent (e.g., a precision of 0.25 would allow an approximate doubling or halving of sample means to be detected). A similar range of precision levels has been used previously for sample size calculations for mosquito populations (Ritchie and Johnson 1991, Zhou et al. 2004) and those of other insects (e.g., Allsopp and Fischer 1999). Calculations were made for predicted means ranging from 1 to 20 *Ae. aegypti* per trap, as this was the range of collection sizes commonly encountered when using BG-Sentinels in Cairns (S.A.R. and C.R.W., unpublished data). The above-mentioned calculations were performed for female *Ae. aegypti* collection data only.

The most appropriate data transformation was determined through the calculation of p using the formula p = 1 − b/2 (Taylor et al. 1978) and previously determined b values. If p = 0, the appropriate transformation for a given data set would be logarithmic, whereas if p = 0.5, a square-root transformation is more appropriate.

Experimental Design for Determining Routine Monitoring Strategy (72-h collections). To determine required sample size, and whether fixed or nonfixed sampling locations should be used, BG-Sentinel trapping was performed at two clusters of houses. To define each cluster, a central house was selected, and all houses within a 75–100-m radius formed the cluster. When permission from residents was refused or the house was unoccupied, the adjacent house was selected. The result was a cluster of almost contiguous houses around the case house; the two clusters were made up of 15 and nine separate residences, respectively.

Each cluster received 16 traps, with one to two traps set at each house, depending on the size of the house and the block of land it was on. Traps were set outdoors in areas sheltered from rain and wind, and they were operated continuously for 15 d, commencing on 6 and 26 May 2005 (late wet season/early dry season) for the two house clusters, respectively. The collection bags were changed every 72 h, providing five time-wise collections from each property (days 3, 6, 9, 12, and 15).

To calculate required sample size for 72-h collections, the same procedure as for 24-h collections was used, i.e., on each sampling date, the s² − m relationship was examined using Taylor’s power law. The resulting a and b values were then used in sample size calculations (Southwood and Henderson 2000) for precision levels from 0.10 to 0.35. Values of a and b
calculated for the two combined house clusters were used to make the sample size calculations as broadly applicable as possible. The calculation of \( p \) as above was used to determine the most appropriate data transformation for 72-h collections. The above-mentioned calculations were made for female \( Aedes aegypti \) collection data.

To determine whether \( Aedes aegypti \) was distributed nonrandomly within a cluster, descriptive statistics (mean, standard deviation, and variance) for total collections (i.e., males and females combined) from 16 traps were calculated for each collection day. The index of dispersion \( (I_d) \) was calculated for each collection day and tested for significance using the chi-square distribution and \( n - 1 \) degrees of freedom (Southwood and Henderson 2000). Significant results \( (P < 0.05) \) indicate deviations from randomness among houses. To determine whether \( Aedes aegypti \) was distributed nonrandomly over time at each house within a cluster, descriptive statistics were calculated for each trap over the 15 d of sampling. \( I_d \) values were calculated for each trap and tested for significance as described above.

Control traps were used to determine whether the continuous operation of BG-SentinelS over 15 d in a cluster of houses was causing localized \( Aedes aegypti \) population reduction. For both clusters, matching controls were operated concurrently at 12 dispersed, noncontiguous houses in the same suburb, \( \approx 700-1000 \) m away from the cluster, for only 24 h at a time every 3 d to coincide with trap collections in the cluster. Thus, controls were only operated for one third of the time at scattered houses, which was thought sufficient for infrequent and dispersed to prevent local \( Aedes aegypti \) population reduction. Time-wise collections from scattered control traps and those in house clusters were tested for significant time–treatment interactions using a repeated measures analysis of variance (ANOVA) procedure with a general linear model in SPSS statistical software, release 11.0.1 (SPSS Inc., Chicago, IL.). Data were log \( (x + 1) \) transformed to normality before analysis. The Premise condition index (PCI) (Tun-Lin et al. 1995) and Breteau index (number of positive containers per 100 houses; BI) were determined for both clusters and their control houses immediately before sampling to determine whether any major disparity in \( Aedes aegypti \) production in treatment and control areas existed.

### Table 1. Required sample size (number of traps) for four levels of precision, \( D \) (standard error/mean), when sampling \( Aedes aegypti \) populations by using BG-SentinelS set for 24 and 72 h

<table>
<thead>
<tr>
<th>Mean/trap</th>
<th>24-h collections</th>
<th>72-h collections</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( D = 0.10 )</td>
<td>( D = 0.15 )</td>
</tr>
<tr>
<td>1</td>
<td>962</td>
<td>138</td>
</tr>
<tr>
<td>3</td>
<td>354</td>
<td>104</td>
</tr>
<tr>
<td>5</td>
<td>234</td>
<td>57</td>
</tr>
<tr>
<td>7</td>
<td>178</td>
<td>37</td>
</tr>
<tr>
<td>10</td>
<td>134</td>
<td>21</td>
</tr>
<tr>
<td>15</td>
<td>96</td>
<td>15</td>
</tr>
<tr>
<td>20</td>
<td>76</td>
<td>12</td>
</tr>
</tbody>
</table>

### Results

**Twenty-Four-Hour Collections for Rapid Abundance Assessment.** In total, 1,905 female mosquitoes were captured in BG-SentinelS set for 24 h. \( Aedes aegypti \) formed 75.4\% of the collections, with \( Culex quinquefasciatus \) Say (17\%) and \( Aedes notoscriptus \) (Skuse) (4.3\%) the other most common species. The mean ± SE 24-h female \( Aedes aegypti \) collection per trap \( (n = 316) \) was 4.5 ± 0.2. Taylor’s power law regressions were all highly significant \( (P < 0.001) \), giving the following values (\( CL_{95} \)): \( \log a = 0.35 \) (0.02; 0.68), \( b = 1.19 \) (0.68; 1.70), and \( r^2 = 0.78 \). These enabled calculation of required sample sizes (Table 1). As the expected mean \( Aedes aegypti \) collection increased, the required number of BG-SentinelS (sample size) decreased for a given precision level. Taylor’s \( p \) (0.41, \( CL_{95} 0.15; 0.66) \) was distinguishable from zero and close to 0.5, indicating that the square-root transformation was most appropriate. To verify this, both square root and logarithmic \( \ln (x + 1) \) transformations were carried out on non-normal female \( Aedes aegypti \) 24-h collection data (seven of 10 collection days). Both transformations normalized all seven data sets as determined by Shapiro–Wilks normality tests in SPSS.

**Seventy-Two Hour Collections for Routine Monitoring.** In total, 652 and 287 female mosquitoes were captured in the respective house clusters. \( Aedes aegypti \) formed 74.2 and 42\% of the mosquito fauna in the two clusters, respectively, with \( Cx. quinquefasciatus \) (16 and 38.3\%) and \( Aedes notoscriptus \) (5.5 and 13.9\%) the other most common species. Linear regressions using Taylor’s power law were all highly significant \( (P < 0.05) \), giving the following values (\( CL_{95} \)): \( \log a = 0.77 \) (0.43; 1.12), \( b = 0.89 \) (0.29; 1.48), and \( r^2 = 0.60 \). Sample size calculations for fixed precision sampling (Table 1) showed that as mean \( Aedes aegypti \) collection increased, required sample size decreased. Increased levels of precision require larger sample sizes. Taylor’s \( p \) was close to 0.5 and distinguishable from zero by 95\% confidence intervals (0.56, \( CL_{95} 0.26; 0.86) \), indicating that the square-root transformation was the most appropriate for skewed data collected using BG-SentinelS set for 72 h. As for the 24-h collections, both square root and log \( (x + 1) \) transformations normalized 72-h collection data sets as determined by Shapiro–Wilks normality tests in SPSS.

Examination of \( I_d \) values (Table 2) revealed a large amount of variation between traps on each collection...
Cluster 2
day 3 15 2.7 6.8 2.6 2.60 75.40 0.00001
Trap 8 5 1.60 1.62 6.70 0.41 14.79 0.0001*
Trap 9 5 0.20 0.45 0.20 2.24 4.00 0.406
Trap 10 5 0.20 0.45 0.20 2.24 4.00 0.406
Trap 11 5 0.20 0.45 0.20 2.24 4.00 0.406
Trap 12 5 0.20 0.45 0.20 2.24 4.00 0.406
Overall 5 0.20 0.45 0.20 2.24 4.00 0.406

Discussion

Required sample sizes for a range of precision levels can be used not only to plan how many BG-Sentinel traps should be set at fixed positions or at randomly reallocated ones, will depend upon the amount of spatial aggregation of Ae. aegypti locally and whether the continuous operation of a BG-Sentinel at a fixed location causes localized trap-down effects. Our studies at the two house clusters revealed

Table 2. Summary statistics of 72-h total Ae. aegypti collections in BG-Sentinel traps set for 15 d in two clusters of houses in Cairo.

<table>
<thead>
<tr>
<th>Cluster 1</th>
<th>Trap 1</th>
<th>Trap 2</th>
<th>Trap 3</th>
<th>Trap 4</th>
<th>Trap 5</th>
<th>Trap 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>3.00</td>
<td>2.30</td>
<td>2.10</td>
<td>3.00</td>
<td>2.30</td>
<td>2.10</td>
</tr>
<tr>
<td>SD</td>
<td>2.57</td>
<td>2.14</td>
<td>1.95</td>
<td>2.57</td>
<td>2.14</td>
<td>1.95</td>
</tr>
<tr>
<td>Variance</td>
<td>6.60</td>
<td>4.56</td>
<td>3.78</td>
<td>6.60</td>
<td>4.56</td>
<td>3.78</td>
</tr>
<tr>
<td>CV</td>
<td>1.22</td>
<td>1.00</td>
<td>0.92</td>
<td>1.22</td>
<td>1.00</td>
<td>0.92</td>
</tr>
<tr>
<td>Id</td>
<td>1.11</td>
<td>0.86</td>
<td>0.80</td>
<td>1.11</td>
<td>0.86</td>
<td>0.80</td>
</tr>
<tr>
<td>P</td>
<td>0.2873</td>
<td>0.1991</td>
<td>0.146</td>
<td>0.2873</td>
<td>0.1991</td>
<td>0.146</td>
</tr>
</tbody>
</table>

Data are sorted to demonstrate variation within a day amongst houses.

Cluster 2

<table>
<thead>
<tr>
<th>Trap 1</th>
<th>Trap 2</th>
<th>Trap 3</th>
<th>Trap 4</th>
<th>Trap 5</th>
<th>Trap 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>3.00</td>
<td>2.30</td>
<td>2.10</td>
<td>3.00</td>
<td>2.30</td>
</tr>
<tr>
<td>SD</td>
<td>2.57</td>
<td>2.14</td>
<td>1.95</td>
<td>2.57</td>
<td>2.14</td>
</tr>
<tr>
<td>Variance</td>
<td>6.60</td>
<td>4.56</td>
<td>3.78</td>
<td>6.60</td>
<td>4.56</td>
</tr>
<tr>
<td>CV</td>
<td>1.22</td>
<td>1.00</td>
<td>0.92</td>
<td>1.22</td>
<td>1.00</td>
</tr>
<tr>
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<td>1.11</td>
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<td>0.80</td>
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<tr>
<td>P</td>
<td>0.2873</td>
<td>0.1991</td>
<td>0.146</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are sorted to demonstrate variation over time for each trap.
that a significant amount of spatial aggregation existed, even between houses in a small cluster. This was consistent with the adult *Ae. aegypti* spatial clustering at the house scale (~10 m) reported by Getis et al. (2003) in Peru; however, the amount of time-wise aggregation at fixed locations in this Cairns study over 15 d was much less pronounced. Furthermore, there was no evidence of localized population reduction through the continuous operation of a BG-Sentinel at a fixed location, suggesting that emigrants from outside the trapping cluster infiltrated the area. These two results indicate that fixed position placement for routine monitoring of *Ae. aegypti* populations in north Queensland is appropriate. Trapping at fixed positions will avoid some of the highly significant variation between nearby houses reported here, and by minimizing variation the ability to detect changes in *Ae. aegypti* abundance is maximized.

The cause of the spatial clustering observed here may be explained in part by the patchy distribution of breeding sites within house clusters. Also, the placement of each trap in proximity to harborage sites for *Ae. aegypti* will affect collections. Thus, spatial patchiness of *Ae. aegypti* production and nonrandom short-range dispersal (Russell et al. 2005) within the cluster are likely contributors to the spatial heterogeneity observed here.

Given that adjacent houses may have vastly different *Ae. aegypti* abundance, indices that are valid at larger scales such as the BI may be of limited importance. The disassociation between houses with high pupal abundance and high adult abundance reported by Schneider et al. (2004) in Peru supports this assertion. *Ae. aegypti* indices based on a smaller scale (e.g., the house unit) may prove more useful in assessing entomological risk. This was well illustrated during a DENV-2 epidemic in Cairns in 2003, when after large-scale vector control operations reduced *Ae. aegypti* collection rates (Ritchie et al. 2004), sporadic transmission continued at previously uninspected houses, each with a small number of breeding sites. The spread of dengue cases was also found to be significantly clustered at the individual house level during an epidemic in Puerto Rico (Morrison et al. 1998).

Although we have provided evidence to guide the sample size and trap placement strategy for rapid abundance assessment and routine monitoring of *Ae. aegypti* populations using BG-Sentinels, the spatial scale over which this should be done has not been assessed here. We have reported spatial clustering at the house scale, which has implications for the number of BG-Sentinels required to make population abundance assessments. However, the spatial scale for rapid abundance assessments, necessary for guiding resource allocation for vector control, will depend upon the spatial distribution of dengue cases, and the short-range dispersal of *Ae. aegypti* in Cairns (Russell et al. 2005). BG-Sentinels can be used to help target source reduction campaigns. Using 24-h collections, traps can be moved daily to help locate households with high immature productivity, especially in cryptic sites. Sciarretta et al. (2005) used such an approach, termed an “Adaptive Population Management Scheme,” to optimize tsetse fly trap locations and control in Ethiopia. However, determining the spatial scale for routine monitoring is more problematic. A detailed spatial analysis of dengue transmission in Cairns that reveals potential “indicator” premises for dengue epidemics should help to clarify this.

Because the breeding ecology of *Ae. aegypti* varies significantly throughout its geographic range, its spatial distribution among houses is also likely to differ between regions. The use of BG-Sentinels as population abundance measures outside of Australia is therefore contingent upon an initial evaluation of spatial patchiness, because this has implications for required sample size (number of BG-Sentinels).

BG-Sentinels have potential as an *Ae. aegypti* population sampling tool, and they provide for the development of an alternative abundance index to the traditional labor-intensive *Aedes* indices for assessing disease risk and the success of vector control programs. However, the relationship between collections of adult mosquito vectors and the amount of disease transmission remains unknown (Focks 2003). It now remains for researchers to determine the relationship between BG-Sentinel collections and dengue infection risk so that epidemiologically relevant *Ae. aegypti* indices can be developed.
Acknowledgments

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