Vector-Borne Diseases, Surveillance, Prevention

OXFORD

Spatial and Temporal Variation in *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) Numbers in the Yogyakarta Area of Java, Indonesia, With Implications for *Wolbachia* Releases

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Abstract

Novel approaches to suppress dengue and other mosquito-borne diseases involve changing the composition of mosquito vector populations, particularly through *Wolbachia* endosymbionts. The success of these strategies depends on understanding the dynamics of vector populations. In preparation for *Wolbachia* releases around Yogyakarta, we have studied *Aedes* populations in five hamlets. Adult monitoring with BioGent-Sentinel (BG-S) traps indicated that hamlet populations had different dynamics across the year; while there was an increase in *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) numbers in the wet season, species abundance remained relatively stable in some hamlets but changed markedly (>2 fold) in others. Local rainfall a month prior to monitoring partly predicted numbers of *Ae. aegypti* but not *Ae. albopictus*. Site differences in population size indicated by BG-S traps were also evident in ovitrap data. Egg or larval collections with ovitraps repeated at the same location suggested spatial autocorrelation (<250 m) in the areas of the hamlets where *Ae. aegypti* numbers were high. Overall, there was a weak negative association ($r < -0.43$) between *Ae. aegypti* and *Ae. albopictus* numbers in ovitraps when averaged across collections. *Ae. albopictus* numbers in ovitraps and BG-S traps were positively correlated with vegetation around areas where traps were placed, while *Ae. aegypti* were negatively correlated with this feature. These data inform intervention strategies by defining periods when mosquito densities are high, highlighting the importance of local site characteristics on populations, and suggesting relatively weak interactions between *Ae. aegypti* and *Ae. albopictus*. They also indicate local areas within hamlets where consistently high mosquito densities may influence *Wolbachia* invasions and other interventions.

Key words: *Aedes aegypti*, *Wolbachia*, population dynamics, Yogyakarta, *Aedes albopictus*

Because of their importance as vectors of dengue, chikungunya, yellow fever, and other viruses, there is a large literature on the ecology and population dynamics of the mosquitoes *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse). *Ae. aegypti* populations are common in highly urbanized environments and particularly inside houses, and their numbers can fluctuate in response to rainfall, temperature, and other environmental variables which appear to be regionally variable (Sheppard et al. 1969, Ho et al. 1971, Chadee 1990, Scott et al. 2000, Barrera 2011). Models that incorporate local rainfall can typically explain a high proportion of the variation in mosquito numbers in some sites (Barrera 2011, Regis et al. 2014), but in other cases temperature seems to be the driving factor (Scott et al. 2000). Locally, *Ae. aegypti* numbers can vary substantially over short distances due to the availability of breeding sites (Edman et al. 1998, Getis et al. 2003) influenced by human behavior (Barrera 2011) and proximity to dense urban areas (Passos et al. 2003, Serpa et al. 2013). Variation in breeding sites across time and space is likely to produce a strong spatial structure of mosquito

density across an area (Jeffery et al. 2009, Hoffmann et al. 2014, Regis et al. 2014).

A substantial amount of information is now also available for *Ae. albopictus*, which tends to be common in rural and suburban areas and has recently spread rapidly around the world (Benedict et al. 2007, Paupy et al. 2009). This species feeds on a wider range of hosts and utilizes a broader range of habitats as immature development sites in urban environments compared with *Ae. aegypti* (Hawley 1988, Hornby et al. 1994) as well as differing in thermal responses from *Ae. aegypti* (Brady et al. 2013). Numbers of *Ae. albopictus* tend to be higher in the wet season and following rainfall events (Tran et al. 2013, Boyer et al. 2014). Where *Ae. aegypti* and *Ae. albopictus* co-occur, there is mixed evidence for competitive interactions among these species (Juliano 1998). Some data and survey results suggest that the species displace each other when they interact, particularly in the case of *Ae. aegypti* being displaced by *Ae. albopictus* in North America (Hobbs et al. 1991, Omeara et al. 1995); however, in many areas, both species appear to persist which may reflect habitat preferences and variation in competitive ability (Passos et al. 2003, Juliano et al. 2004, Serpa et al. 2013). Overall, these previous studies point to the importance of regional variation in mosquito dynamics reflecting local weather conditions, human behavior, the extent to which environments are urbanized, and species interactions.

In this paper, we provide information on the local dynamics of *Ae. aegypti* and *Ae. albopictus* in five hamlets around Yogyakarta, Indonesia. Previous research on these mosquitoes have suggested seasonal patterns in changes in mosquito numbers associated with rainfall (Wai et al. 2012). In the dry season, populations may partly contract to wells (Gionar et al. 1999), although there are likely to be many cryptic breeding sites. The number of coexisting *Ae. albopictus* are low in the suburban areas but increase in villages around the city (Umniyati 1992, Umniyati and Umayah 1992). We have collected these data in preparation for releases of mosquitoes carrying the *w*Mel strain of *Wolbachia* bacteria in Yogyakarta, following successful releases aimed at *Wolbachia* invasion by this strain in northern Australia (Hoffmann et al. 2011).

Our aim is to collect data to assist in the design of *Wolbachia* mosquito release strategies. We tackle four main questions. 1) Based on adult trapping and ovitraps, how do numbers of *Ae. aegypti* and *Ae. albopictus* change across time at each hamlet, and to what extent are these changes predictable? 2) What is the fine-scale distribution of *Ae. aegypti* and *Ae. albopictus* within the potential release areas and how stable is this distribution particularly across seasons? 3) Do adult trapping and ovitraps indicate a similar spatial pattern and how might this inform future surveys? 4) Does the effectiveness of ovitrapping depend on whether traps are placed inside and outside dwellings and to what extent are these correlated? 5) Is there evidence for hotspots in terms of high mosquito numbers collected in traps and do these persist across time and the wet and dry seasons?

Materials and Methods

Sites

Five hamlets were selected around the Yogyakarta city area (Fig. 1), consisting of 300–1,200 houses. Jomblangan (covering 0.28 km^2, 672 houses, density 9,050 residents/km^2) and Singosaren (0.145 km^2, 311 houses, 7,895 residents/km^2) are located in the Bantul District, while Nogotirto (0.286 km^2, 770 houses, 9,374 residents/km^2), Trihanggo (0.515 km^2, 1,115 houses, 7,076 residents/km^2),

and Kronggahan (0.392 km^2, 797 houses, 6,927 residents/km^2) are located in the Sleman District. Hamlets were selected based on a high level of community participation and the fact that they were separated physically from other hamlets, making them suitable for initial *w*Mel *Wolbachia* releases. Hamlets consist of areas of dense housing separated by pockets of vegetation, which makes up 30–50% of the hamlet area. Temperature in the region does not vary seasonally and averages 26.4°C, while December–March are the wettest months. Rainfall data accumulated over 24 h were collected daily from each hamlet.

Adult *Aedes* were monitored with BioGent-Sentinel (BG-S) traps (Biogents AG, Regensburg, Germany) without lures placed around each hamlet. These traps were connected to a mains power supply but were also fitted with a novel battery back-up system that allowed for continuous operation when the mains supply was interrupted. The battery was of type LC-V127R2NA providing 13.6–13.8 V, with a capacity of 7.2 Ah/20 HR. Numbers of BG-S traps per hamlet varied between 11 (at Singosaren, the smallest site with the fewest houses) and 13 (at the larger sites including Trihanggo and Kronggahan). BG-S were set up in corners of rooms indoors on May 2012 and monitored until the start of 2014 in the same place. Traps were active throughout this period. Samples were collected every week and identified for *Aedes* species based on characteristic morphology.

Ovitraps provided a way of monitoring *Ae. aegypti* and *Ae. albopictus* distributions at a finer spatial scale. Each ovitrap consisted of a red bucket 12.5 cm in diameter and 13 cm in height. About 900 ml water was added to a level of 9 cm along with an oviposition strip (red cloth, 5 by 12 cm^2) attached to the side of the bucket and suspended partly in the water. Fish food (Pleco Wafers, Melie, Germany) was added as an attractant. The ovitraps were placed indoors and outdoors (one in each location) at around 100 randomly selected houses in each site (i.e., 200 traps in total). All ovitraps were retrieved after 7 d and transported to an insectary, where eggs were counted. Strips with eggs were dried and incubated for 3 d, then individually placed into plastic containers for hatching under ambient conditions. Twenty-five fourth-instar larvae were selected and identified from each container. Identification was based on the presence of the metathoracic spine and toothed comb scales. Ovitrapping was carried out between November 2012 and July 2014 on 10–11 occasions per site, with traps placed in the same position on each occasion.

Analysis

We used the weekly BG-S data to assess changes in adult populations over time and to test for links between these changes and local rainfall patterns. BG-S data were sorted into *Ae. aegypti* and *Ae. albopictus* as well as other species (mostly *Culex*), and numbers of *Aedes* species compared across time when expressed as the mean numbers of females and males collected in traps per week. To examine temporal changes in mosquito numbers (pooled across the sexes, although analyses with only females produced similar results), we initially tested for autocorrelation using IBM SPSS Statistics 20. Consistent with previous surveys (e.g., Regis et al. 2014), we found that mosquito numbers were autocorrelated with a lag of 1 wk at the different sites. We then ran an autoregressive model (AR1) to examine the association between rainfall and BG-S counts. The rainfall (which had been accumulated daily) was summed over the previous 2 wk, 1 mo, and 2 mo before each BG-S sample had been collected. Different time periods were used to reflect short-term effects of rainfall (where rainfall events trigger the completion of
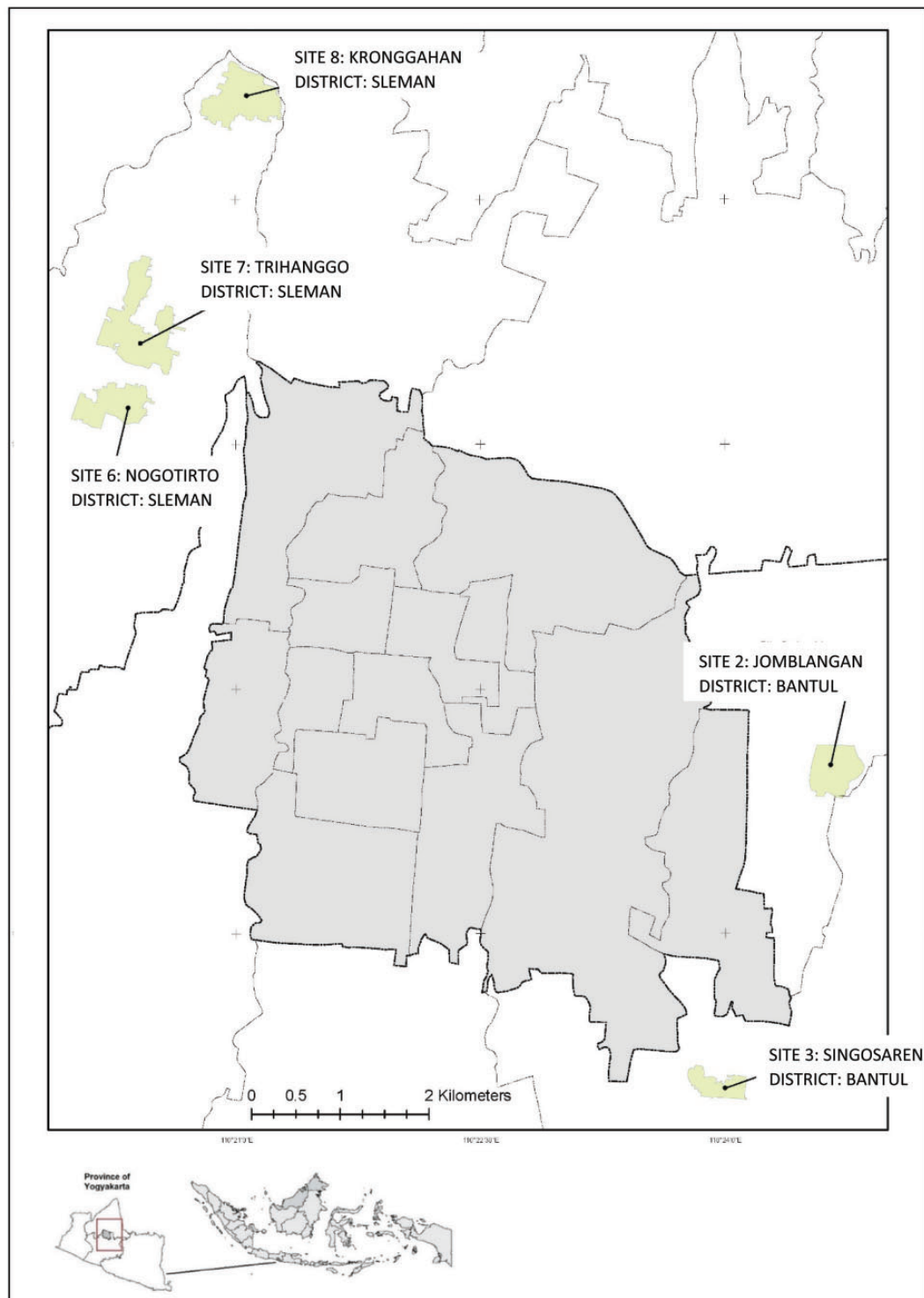
Fig. 1. Map of Yogyakarta showing city area and five hamlets around the city where monitoring took place.

larval development such as through the introduction of food into breeding containers) as well as longer term effects promoting new breeding cycles.

The ovitrap data were used to assess population numbers and were also linked to BG-S counts to test whether these trapping methods provided similar types of information on overall population numbers. We averaged BG-S counts obtained in the same week that ovitrap data were collected, as well as in the week prior to the

collection and in the two weeks following the collection, and then correlated these measures. Because mostly *Ae. aegypti* were collected in the BG-S traps, we only considered this analysis for *Ae. aegypti* and averaged ovitrap numbers inside and outside houses.

Spatial patterns within sites were investigated by considering ovitrap counts from a particular location within a hamlet averaged across trapping events. We initially correlated two indices of mosquito numbers, involving the proportion of traps that were positive

for a species or the estimated number of eggs of a species (c.f. Regis et al. 2014). Both these indices provide information about mosquito numbers in a population. The number of eggs of a species was estimated from the egg count on the oviposition substrate of an ovitrap multiplied by the proportion of eggs estimated to belong to that species based on the hatched larval identifications. Because these indices were always highly correlated (Supp Fig. 1 [online only]), further analyses were only carried out using the index based on egg counts which uses more information than the presence or absence based index.

We estimated spatial structure within hamlets based on the ovitrap data by computing Moran's I with the SAM (Spatial Analysis for Macroecology) program (Rangel et al. 2010) and significance of spatial patterns established through randomization. We then assessed whether there was a correlation between trap numbers collected inside and outside houses (*Ae. aegypti* only) based on correlation coefficients without considering spatial structure and then correcting for spatial structure using the Dutilleul approach (Dutilleul 1993). The association between *Ae. aegypti* and *Ae. albopictus* numbers in ovitraps placed outside houses (where this species was mostly found) was also investigated in this way.

To identify whether *Ae. aegypti* and *Ae. albopictus* numbers were related to vegetation versus the built area around a location where ovitraps were placed, we used ArcGIS to estimate the proportion of a 25-m circle drawn around the ovitrap position consisting of built-up area (mostly housing) versus vegetation as assessed from satellite images obtained in Google Earth and then mapped. Small proportions of areas were also assigned to roads and agricultural fields but these were not included in the analyses. We then examined the association between vegetation and the numbers of mosquitoes retrieved from traps outside houses averaged across collections. We also examined these associations in BG-S traps, but data were only available for 11–13 traps placed in each hamlet in contrast to 100 ovitraps. For the BG-S traps, we considered built area and vegetation within a 100-m radius of each trap to reflect the larger area expected to be sampled by these traps (e.g., Azil et al. 2014).

Finally, to test for hotspots where there were consistently high (or low) mosquito numbers within a site, we used the Getis-Ord Gi* statistic with a Euclidean distance computed from average mosquito densities in ovitrap sites based on the egg or larval counts. We ran this analysis in ArcGIS with and without an adjustment for the false discovery rate (FDR). We also computed correlations between ovitrap counts (based on egg or larval data, inside and outside each house combined) averaged across collections in the first dry season (2012), the 2012–2013 wet season, and the second dry season (2013). We computed Spearman rank correlations (r_s) because data were skewed. These correlations indicate whether areas with high ovitrap counts tended to be consistent across time.

Results

Changes in Counts Across Time

Individual weekly BG-S counts were positive for *Ae. aegypti* on most occasions, ranging from 78.7% in Kronggahan to 89.9% in Jomblangan. The average counts across weeks indicate contrasting dynamics at the five sites (Fig. 2). For two of the sites (Kronggahan and Trihanggo) on the east side of Yogyakarta city where human population densities were relatively lower, numbers were relatively constant throughout the sampling period except for a modest increase in the November–March wet season ($\bar{x} = 5.68 \pm 0.32$ (SE) in November 2012 to March 2013, compared with $\bar{x} = 4.50 \pm 0.22$

in April 2013 to August 2013; *t* test, $P = 007$ for Kronggahan and $P = 0.132$ for Trihanggo). Numbers at Trihanggo tended to be higher than at Kronggahan in most trapping events (68%, paired *t* test, $P < 0.001$). On the other hand, numbers at Jomblangan and Nogotirto (where human population densities were higher) and to a lesser extent Singosaren tended to fluctuate, peaking during the wet season (Jomblangan, $\bar{x} = 11.72 \pm 1.18$; Nogotirto, $\bar{x} = 10.36 \pm 1.00$; Singosaren, $\bar{x} = 6.26 \pm 0.44$), and returning to levels comparable with other hamlets during the drier period ($\bar{x}$ 6.34, 4.92, 5.45 for these sites, respectively; *t* tests, $P < 0.001$ except for Singosaren, $P = 0.021$). These sites are all on the west side of Yogyakarta and suggest different dynamics on the two sides of the city. The number of *Ae. albopictus* retrieved from the BG-S traps was relatively low (Fig. 2), making seasonal patterns and site differences difficult to discern. Nevertheless there was an increase in numbers in all hamlets during the wet season ($\bar{x}$ 0.42 ± 0.072) compared with the dry season $\bar{x}$ 0.35 ± 0.053) and particularly at the end of 2013 when numbers caught averaged > 1.

For the ovitrap data, the percentage traps positive for *Ae. aegypti* varied from 20% to around 70%. Abundance of *Ae. aegypti* estimated from ovitrap data (averaged across traps placed inside and outside houses) tended to match differences across seasons and hamlets evident from the BG-S traps (moving average over the 4 wk around the ovitrap event), regardless of whether ovitrap data were scored based on eggs and larvae or presence or absence data (Fig. 3a and b). The positive correlations between counts obtained with these trapping methods (egg or larvae data, $r = 0.746$, $P < 0.001$, $N = 38$; presence or absence data, $r = 0.697$, $P < 0.001$, $N = 38$) suggest that either method can be used to estimate the relative abundance of *Ae. aegypti* across hamlets and trapping times. The positive relationship between these trapping methods was evident in the dry ($r = 0.761$) as well as the wet ($r = 0.688$) seasons.

The total accumulated daily rainfall each week in each hamlet tended to be correlated with rainfall in other hamlets. Spearman rank correlations varied from high values of 0.88 (Nogotirto and Singosaren) and 0.85 (Jomblangan and Singosaran) to a low value of 0.52 (Trihanggo and Jomblangan), suggesting that rainfall events were patchy across the area where the hamlets were located and different particularly between the two districts on opposite sides of the city.

In all hamlets, BG-S counts were positively autocorrelated, with a lag of 1 or 2 wk depending on the hamlet (1 wk for Jomblangan and Kronggahan, 1 and 2 wks for the other sites: Table 1). A regression analysis with AR1 models indicated that rainfall in the month immediately preceding the BG-S collection associated most strongly with BG-S counts in all sites (compared with rainfall in the preceding 2 wk or 2 mo) except at one hamlet (Jomblangan) where the association was relatively stronger for rainfall across the preceding 2 mo (Table 1). Once the 1-mo (or 2-mo in the case of Jomblangan) rainfall was included in the autoregressive model, no other variables were significant. The association between rainfall in the previous month and weekly BG-S counts (log transformed; Fig. 4) was always positive, with R^2 values in the range 0.12–0.31 ($P < 0.01$ in each case). These patterns suggest that rainfall triggered egg hatching and increased egg production and stimulated the development of larvae already present in containers, leading to a higher emergence rate of adults.

Variation in Mosquito Numbers Within Sites

Ovitrap data (averaged across collections) were used to investigate spatial structure. Ovitraps were included when they produced data
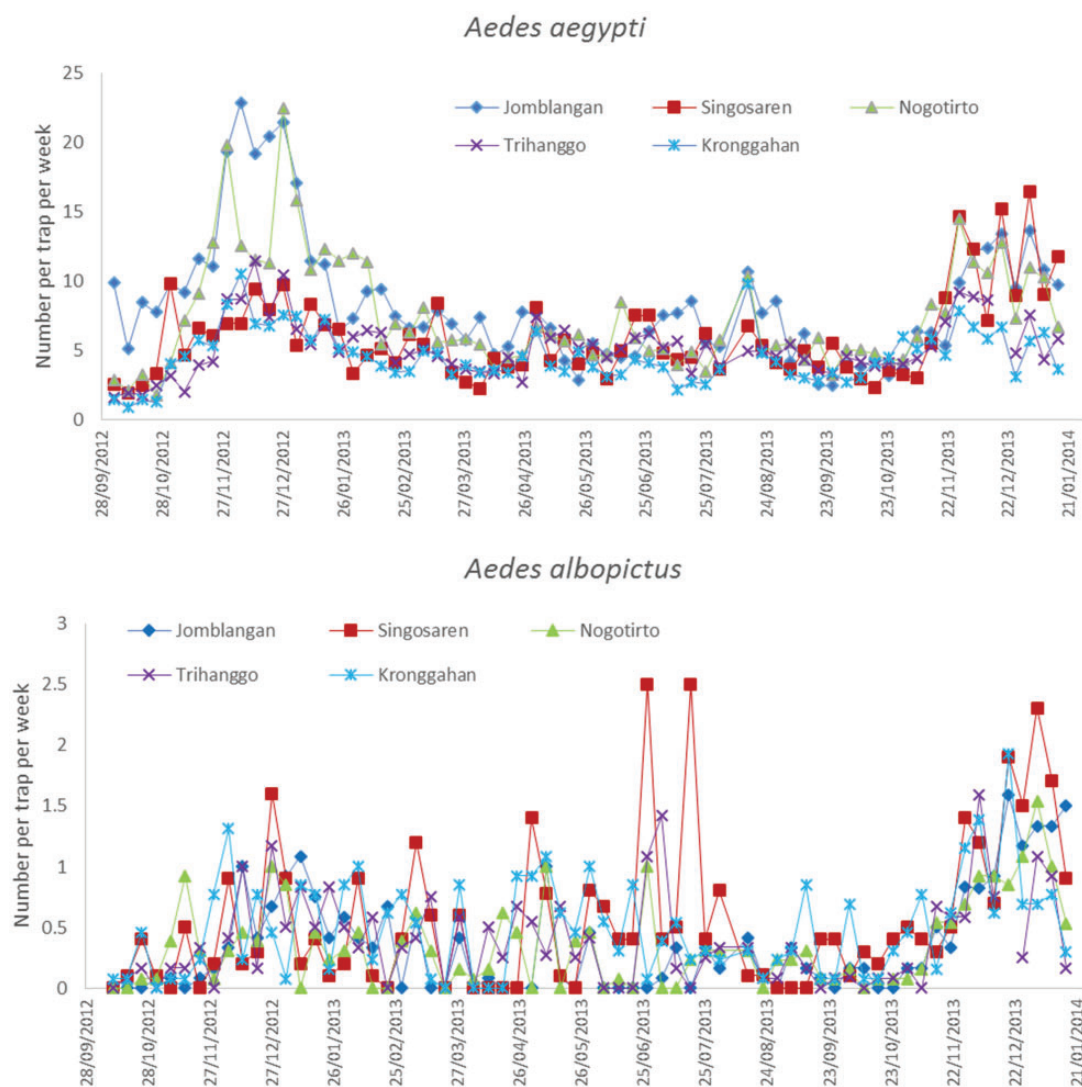
Fig. 2. Changes in mean weekly BG-S counts of *Ae. aegypti* and *Ae. albopictus* across time at the five hamlets. Numbers of females and males are combined and averaged across the 11–13 traps per location.

(including negative data) on all trapping occasions, resulting in 82 to 105 ovitraps being included per site. Significant spatial structure (as defined by Moran's I) in the ovitrap data was detected for *Ae. aegypti* trapped inside houses at Singosaren, Trihanggo, and Kronggahan where human population densities tended to be lower. At the first two sites and based on Moran's I, significant structure was restricted to the first distance class (50 m and 80 m at Singosaren and Trihanggo, respectively), while at Kronggahan it involved distances out to 190 m (Supp Fig. 2 [online only]). For *Ae. aegypti* outside houses, there was structure at Nogotirto (distance to 130 m), Trihanggo (distance to 240 m), and Kronggahan (70 m only; Supp Fig. 2 [online only]). Finally for *Ae. albopictus* collected outside houses, there was structure only at Singosaren (distances to 90 m).

We also used averaged ovitrap data to consider associations between mosquito numbers and both vegetation or buildings (within 25-m circles) and distance to hamlet edge. Here, we only present patterns for vegetation (rather than built-up area) because the proportion of a circle consisting of vegetation versus buildings (ignoring agricultural areas and roads) was always strongly negatively correlated ($r < -0.9$). For *Ae. aegypti*, there was a negative association

between ovitrap numbers and percent vegetation, although this pattern was weak at some sites (particularly Nogotirto; Fig. 5). After spatial correction, correlations between vegetation and numbers of *Ae. aegypti* were significantly negative for Jomblangan ($r = -0.296$, $P = 0.011$), Singosaren ($r = -0.270$, $P = 0.020$), and Trihanggo ($r = -0.305$, $P = 0.024$). There were positive associations between vegetation and the abundance of *Ae. albopictus* (Fig. 5). Correlations between vegetation and *Ae. albopictus* numbers were only weakly affected by spatial correction; for Jomblangan, Nogotirto, Trihanggo, correlations were 0.394, 0.389, and 0.365, respectively, and these were significant at the $P = 0.001$ level with and without spatial correction. For Singosaren and Kronggahan, the correlations were 0.091 and 0.220, respectively, and these were not significant after correction for spatial patterns ($P = 0.428$ and 0.067, respectively). There were no associations between mosquito numbers and the distance of traps from the edge of hamlets (all with $P > 0.10$).

We also considered the association between numbers of *Ae. aegypti* found inside and outside houses when averaged across the ovitrap collection periods. These associations were all positive (Fig. 6) although perhaps weaker than might be expected given that
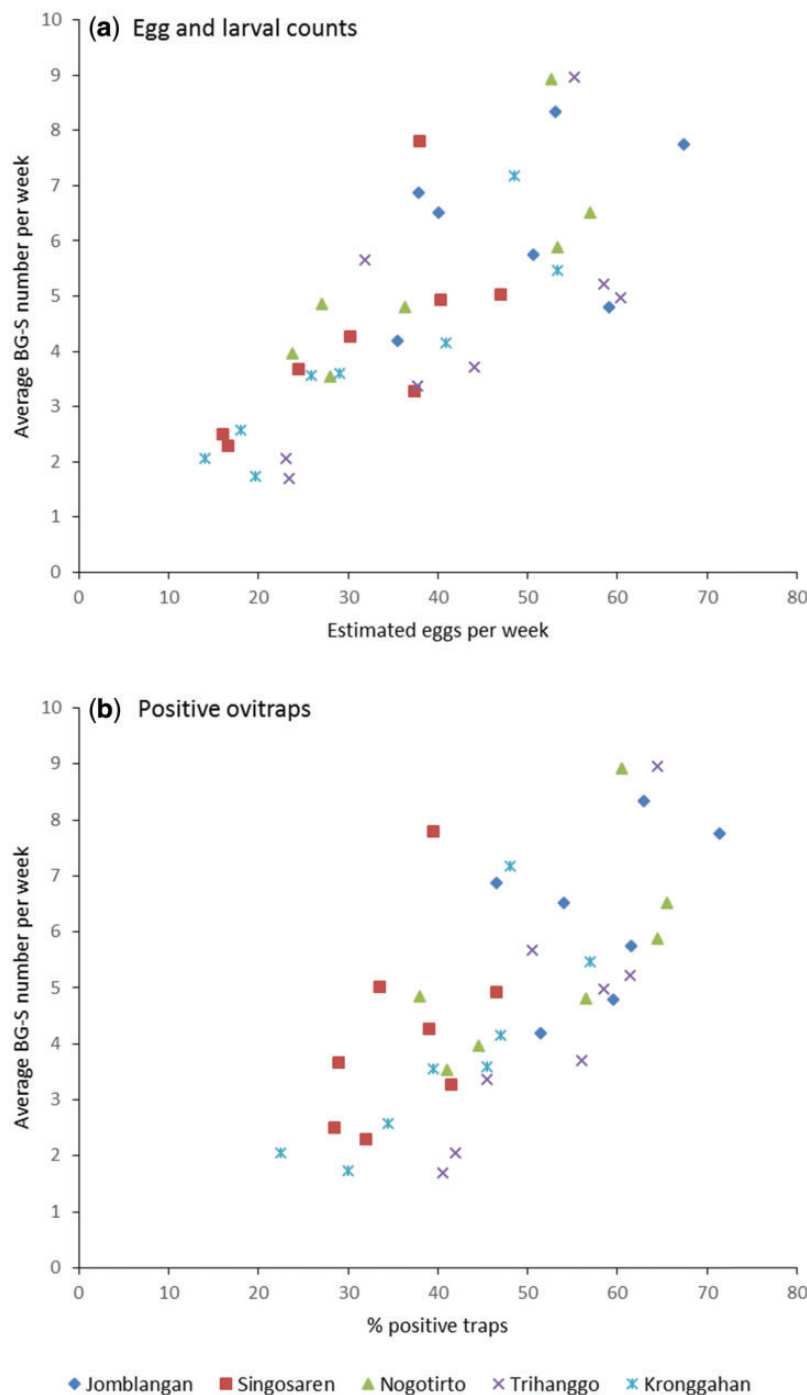
Fig. 3. Comparison of BG-S and ovitrap numbers for *Ae. aegypti*. BG-S numbers were averaged 2 wk before and after each period that ovitraps were out (i.e., 4 wk in total). Ovitrap numbers represent *Ae. aegypti* estimates based on (**a**) egg and larvae numbers averaged across traps and (**b**) presence or absence of *Ae. aegypti* recorded in the traps.

ovitraps were placed inside and outside the same residences. For Jomblangan, Singosaren, Nogotirto, Trihanggo, and Kronggahan, correlations were 0.460, 0.452, 0.256, 0.349, and 0.452, respectively ($N = 82–105$). All correlations remained significant at the 0.01 level after correction for spatial patterns with the exception of Nogotirto which had an uncorrected P value of 0.015. We did not consider the association between traps inside and outside houses for *Ae. albopictus* because of the relatively low abundance of *Ae. albopictus* caught inside houses (on average per site, the number of this

species collected outside was two to five times greater than inside a house, with an overall mean of 0.081 ± 0.001 inside houses and 0.205 ± 0.001 outside houses).

To look for the presence of areas with consistently high or low *Ae. aegypti* mosquito densities across time within a site, we computed rank correlation between ovitrap counts from three collection periods in the dry (2012), wet (2012–2013), and dry (2013) periods. All 10 correlations between collections adjacent in time were positive (r_s in the range 0.08 to −0.48, 5 hamlets, two correlations per

Table 1. Autoregressive models linking rainfall (in preceding 1-monthly or 2-monthly period) to average weekly BG-S counts at the different sites

Site	Variable	Estimate	SE	t	P
Jomblangan	Constant	1.539	0.151	10.199	<0.001
	Lag (1)	0.523	0.118	4.444	<0.001
	2-monthly rainfall	0.081	0.028	2.905	0.005
Singosaren	Constant	1.337	0.155	8.639	<0.001
	Lag (1)	0.137	0.125	1.097	0.278
	Lag (2)	0.472	0.124	3.789	<0.001
	1-monthly rainfall	0.176	0.050	3.526	0.001
Nogotirto	Constant	1.546	0.157	9.857	<0.001
	Lag (1)	0.310	0.134	2.305	0.025
	Lag (2)	0.255	0.142	1.796	0.078
	1-monthly rainfall	0.151	0.051	2.968	0.004
Trihanggo	Constant	1.487	0.113	13.174	<0.001
	Lag (1)	0.201	0.130	1.553	0.126
	Lag (2)	0.378	0.138	2.728	0.009
	1-monthly rainfall	0.059	0.035	1.676	0.100
Kronggahan	Constant	1.222	0.111	11.043	<0.001
	Lag (1)	0.494	0.121	4.084	<0.001
	1-monthly rainfall	0.110	0.039	2.838	0.006

Autoregressive integrated moving average models were fitted ([1, 0, 0] or [2, 0, 0]) depending on whether 1- or 2-wk lags were identified in an initial autocorrelation analysis. Rainfall parameters and SEs are multiplied by 100. Significant P values are highlighted.

hamlet) and seven were significant, reflecting the fact that traps with high numbers in one period also tended to have high numbers in the ensuing period. We undertook analyses of hot spots and cold spots based on ovitrap data averaged across the entire period. For all sites, hot spots and cold spots were identified (Supp Fig. 3 [online only]) which remained evident for all hamlets after FDR correction based on the Getis-Ord Gi* statistic. Cold sites tended to be peripheral to the hamlets, while hot sites were more centrally located.

Finally, with respect to numbers of *Ae. aegypti* and *Ae. albopictus*, we considered the association between these species in outside traps when both species were present in the traps (to exclude sites where conditions may have been unsuitable for one of the species, such as a complete absence of vegetation cover which often led to the absence of *Ae. albopictus*). In these analyses, the abundance of species tended to be negatively associated (Supp Fig. 4 [online only]). Correlations were significant after spatial adjustment for Singosaren ($r = -0.327$, $P = 0.008$), Nogotirto ($r = -0.297$, $P = 0.036$), Trihanggo ($r = -0.387$, $P = 0.003$), and Kronggahan ($r = -0.420$, $P = 0.002$) based on sample sizes of 57–76 traps.

For the BG-S counts, there were only a limited number of traps to consider spatial patterns within a site. Nevertheless, we did consider the association between vegetation cover and numbers of *Ae. albopictus* collected from the traps. In this case, percent vegetation was computed from 100-m circles around the traps given that these traps were expected to collect mosquitoes from a wider area. For these data, patterns were consistently positive across the sites (Supp Fig. 5 [online only]), although because of low trap numbers ($N = 11$–13 per hamlet) the correlation was only significant for Nogotirto ($r = 0.789$, $P = 0.001$, $N = 13$).

Discussion

The findings suggest that the dynamics of *Ae. aegypti* and *Ae. albopictus* populations as assessed through adult collections and oviposition behavior differ between hamlets around Yogyakarta. Each site we surveyed is to some extent affected by local dynamics given that the patterns across the year varied across hamlets, with sharper changes in abundance at some sites and weaker changes at others. Previous research in areas including Yogyakarta have noted the effect of rainfall patterns on *Aedes* abundance (e.g., Wai et al. 2012) and rainfall did vary across the hamlets. However, rainfall only accounted for some of the variation in mosquito numbers with low to moderate R^2 values. Apart from rainfall, there are likely to be other factors influencing mosquito abundance such as the nature of breeding sites in the different areas; these might vary seasonally due to factors like changing levels of water in wells. However, in the absence of specific knowledge about many of the breeding sites in this region apart from bak mandi sinks, tires, and wells (Gionar et al. 1999, Focks et al. 2007, Mardihusodo et al. 2011), it is difficult to predict factors influencing the local dynamics of the mosquito populations.

The seasonal patterns noted for *Ae. albopictus* in Yogyakarta fit those seen at other sites. *Ae. albopictus* was relatively common in all hamlets and particularly in the wet season in traps outside houses. This pattern has been noted in other locations such as Thailand and Brazil (e.g., Mogi et al. 1988, Honorio et al. 2009) and can lead to the apparent replacement of *Ae. albopictus* by *Ae. aegypti* across a year as in the Philippines (Duncombe et al. 2013). However, these patterns do not necessarily imply strong competitive interactions between the species (Juliano 1998). *Ae. albopictus* may appear to be at a competitive advantage over *Ae. aegypti* in some situations such as when there is an abundance of leaf litter and resources are limiting (Juliano 1998, Braks et al. 2004, Juliano et al. 2004) and this might contribute to the consistent but weak association we found between *Ae. albopictus* numbers and vegetation. The relatively higher abundance of *Ae. albopictus* in areas where vegetation occurs may also reflect the broad range of natural and artificial containers that this species utilizes as immature development sites, including tree hollows, broken bamboo stems, and other sites in vegetation where water accumulates (Hawley 1988). Both competition and breeding site preference could contribute to the negative association of the two species in trap locations where the species co-occurred.

The data we have collected point to both ovitraps and BG-S traps being suitable for monitoring mosquito populations, given that hamlet variation and seasonal differences in mosquito numbers estimated through these measures showed similar patterns. BG-S traps collect adults in the vicinity of the trap and any mosquitoes moving through premises and have previously been used in assessing population size at a local scale (Johnson et al. 2012, Ritchie et al. 2013). An advantage of BG-S traps is that they can be readily serviced by field staff because the traps can be left in position when recovering adults, particularly when traps are left in the same location for an extended period as in the current surveys. With the novel battery backup installed in the BG-S traps, the traps provided an ongoing monitoring system. However, BG-S traps are expensive and rely on cooperative home owners and secure locations. Ovitraps have also been widely used in surveillance of mosquito populations and can provide effective tools with adequate sampling (e.g., Mogi et al. 1988, Lee 1992, Mackay et al. 2013). They are cheap to produce and can provide a high level of spatial resolution of local mosquito densities (Mackay et al. 2013, Hoffmann et al. 2014). Our data also indicate that ovitraps can provide effective monitoring even when they are only scored for presence or absence of mosquitoes. Because only a few females contribute eggs to ovitraps (Hoffmann et al. 2014), there may be limited benefit in scoring tens of larvae from
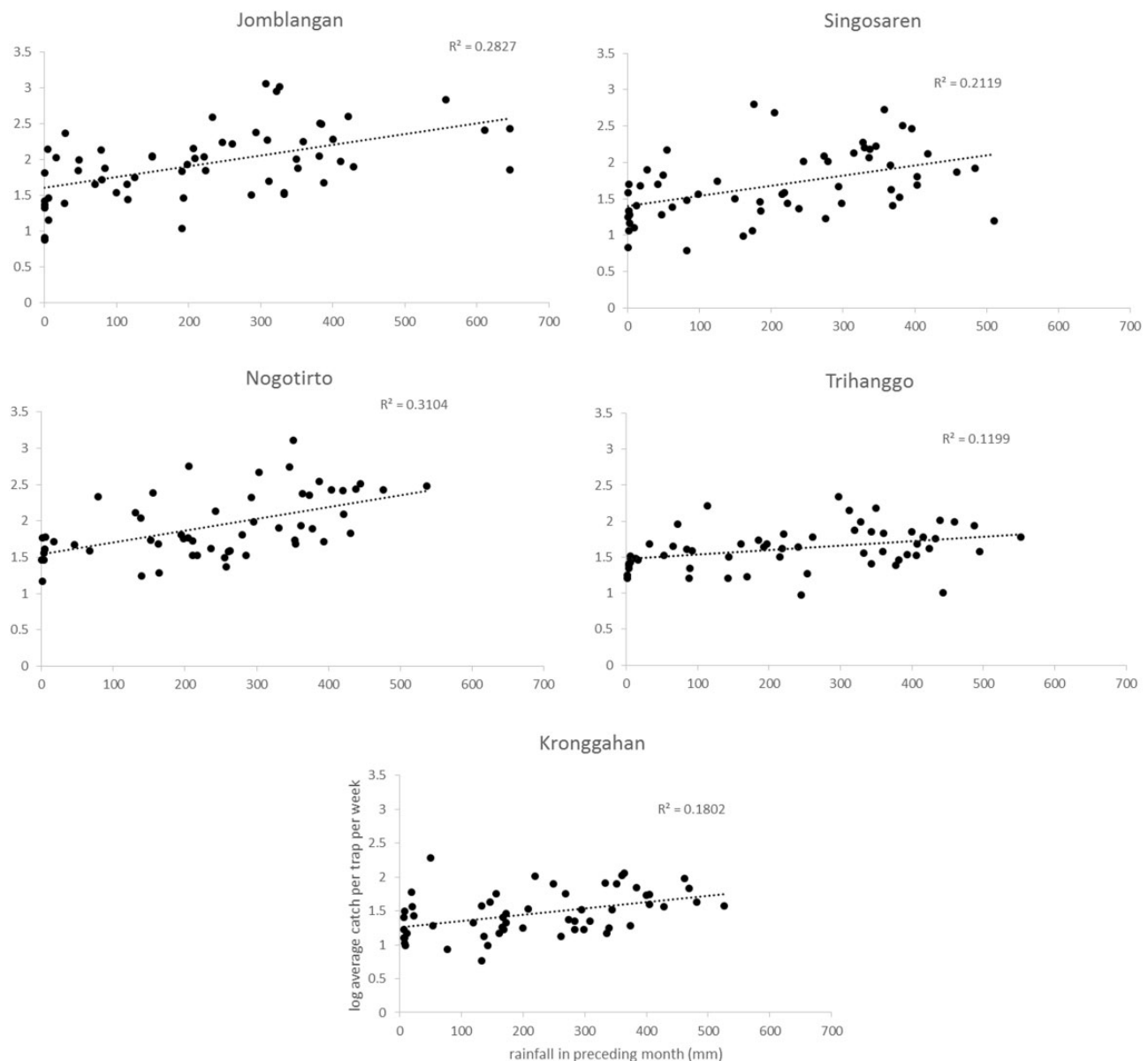
Fig. 4. Effect of preceding 1-mo rainfall on weekly BG-S counts of *Ae. aegypti* at the five hamlets. BG-S counts represent log transformed numbers of males and females (summed). R^2 values from linear regressions are indicated.

individual ovitraps rather than using a simple presence or absence measure. It remains to be seen if these types of traps given comparable results in areas where mosquito densities are low, such as in Australia. However, in the Yogyakarta sites the relationship between trapping methods held in the dry season when mosquito numbers were lower.

Although these results provide information on the utility of different trapping methods and point to associations between mosquito numbers and environmental attributes, our study also has some limitations and highlights the need for additional work. In particular, we did not consider associations with other measures of mosquito activity in residential areas that are used by researchers and agencies, such as indices that measure breeding containers around houses. We were also unable to consider other important variables expected to affect changes in mosquito numbers, such as chemical control programs that target adults through fogging or larval breeding sites. Future studies might consider these issues along with a

proper cost-benefit analysis associated with different monitoring methods, and encompass a longer time frame so that seasonal changes and hamlet variation can be validated across a number of years.

Implications for *Wolbachia* Replacement Releases

During *Wolbachia* replacement releases in mosquitoes, an accurate assessment of *Wolbachia* frequencies in natural populations is needed to track the success of an invasion, and an estimate of infected and uninfected adults is needed so that release numbers can be adjusted to exceed the threshold required for invasion (Turelli 2010, Hoffmann et al. 2011, Ritchie et al. 2013). During releases, ovitraps have the advantage in providing accurate *Wolbachia* frequency estimates because these traps collect ovipositing females that have already secured a bloodmeal and are therefore able to contribute to the natural population (Yeap et al. 2013). In contrast, adults
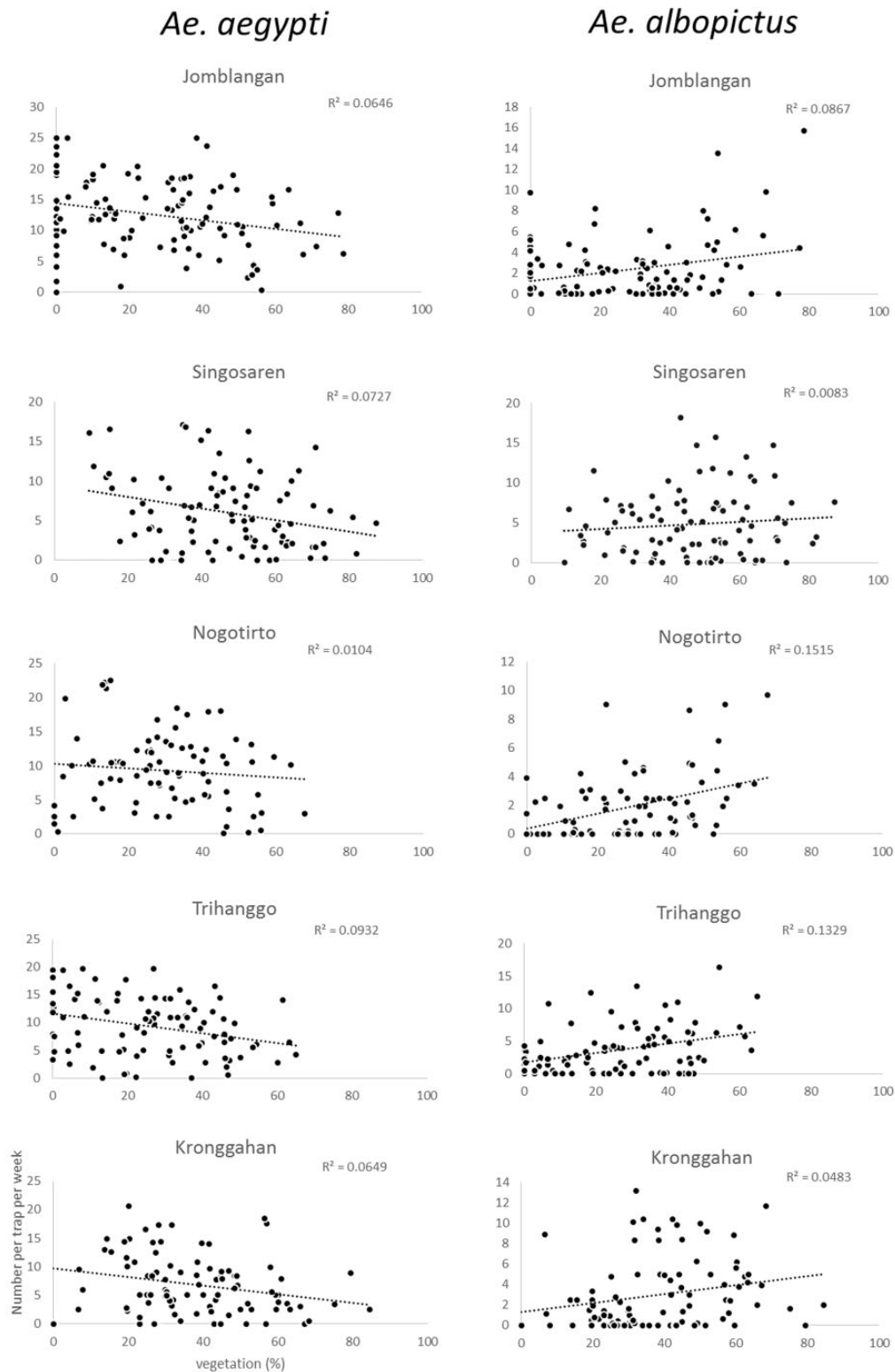
Fig. 5. Associations between percent vegetation in the 25-m area around an ovitrap (x axes) and estimated average egg counts (y axes) of *Ae. aegypti* and *Ae. albopictus* in ovitraps placed outside houses.

from BG-S traps will include mosquitoes that have been artificially reared and released and might not contribute offspring. On the other hand, during *Wolbachia* releases, BG-S traps have the advantage of providing continuous monitoring of adults with little effort because traps remain in situ, and changes in infected and uninfected adult numbers can be used to directly estimate population size (Ritchie et al. 2013). Moreover, BG-S traps collect adults that can then be screened for viruses as well as *Wolbachia*, an important
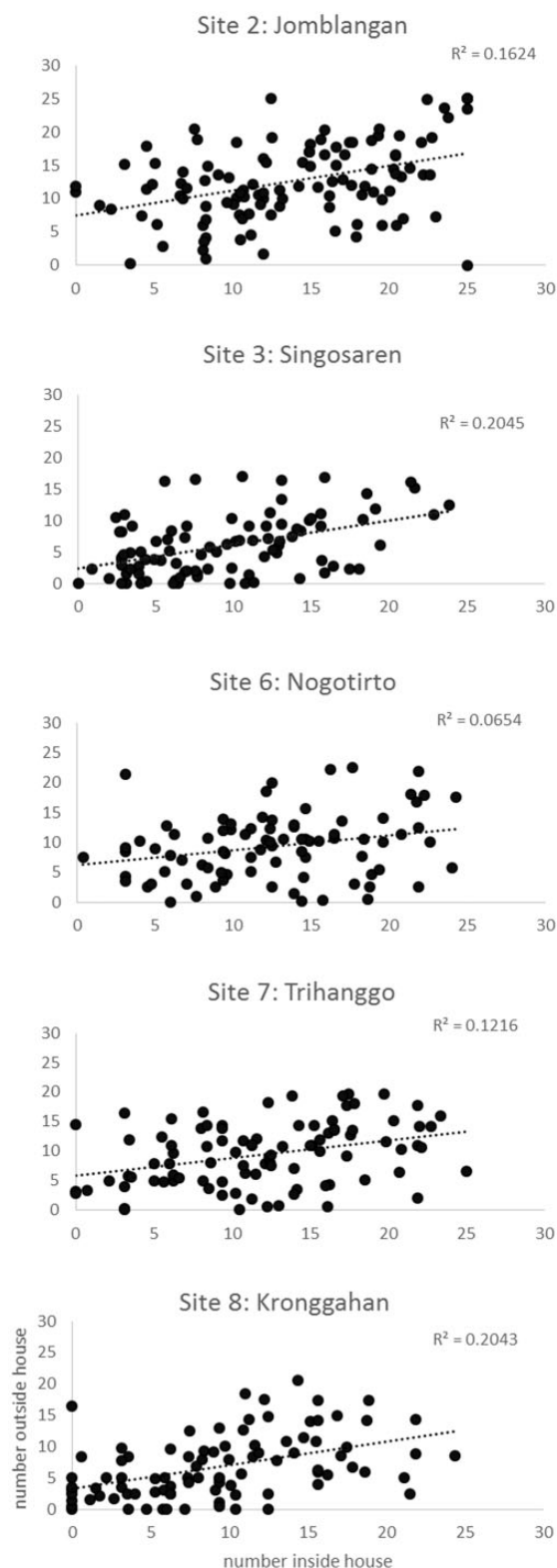
Fig. 6. Association between estimated *Ae. aegypti* egg numbers for ovitraps placed inside and outside houses. Numbers are averaged across trapping collections.

consideration when assessing the impact of *Wolbachia* on dengue transmission by mosquitoes (Ferguson et al. 2014). A combination of trapping methods may therefore be useful for ongoing monitoring during releases in Yogyakarta and other locations.

The current results suggest BG-S counts in the range of 5–15 per week at most sites (combined sexes) which is at the upper end of the numbers present in BG-S traps in Australian releases (c.f. Hoffmann et al. 2011). Assuming that numbers in BG-S traps in Yogyakarta are comparable with those collected in northern Australia, this would suggest a density of 5–10 females per residence (Ritchie et al. 2013). However, such numbers will need to be adjusted because of differences between release sites in terms of the density of people per residence and area occupied by individual residences. Once releases are initiated, baseline data such as those collected here can be used to provide an accurate estimate of population size (Ritchie et al. 2013).

Finally, the data collected here point to the possibility of areas within hamlets where mosquito densities are consistently high. This can slow rates of local invasion as has occurred in the invasion of *Wolbachia* into Australian sites such as in the case of the invasion of Gordonvale by the *w*Mel strain of *Wolbachia* (Hoffmann et al. 2014). Areas with high mosquito densities can be identified through ovitrapping as undertaken here, and the Yogyakarta data suggest that these areas may extend to 200 m from a trap. If invasion proves difficult in some regions within a hamlet, it might then be possible to increase release rates in such areas, a process of "adaptive management" that has previously been applied in releases in northern Queensland (Hoffmann et al. 2011).

In conclusion, the entomological data collected from hamlets around Yogyakarta have highlighted the usefulness of both BG-S traps and ovitraps in monitoring changes in *Ae. aegypti* and the distribution of this species relative to *Ae albopictus* across hamlets. We have identified areas where numbers of mosquitoes are consistently high, and some of the features influencing the abundance of the mosquitoes including vegetation. This information should be useful in *Wolbachia* release strategies aimed at invading *Wolbachia* across release sites or other approaches aimed at suppressing populations.

Supplementary Data

Supplementary data are available at *Journal of Medical Entomology* online.

Acknowledgments

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