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Characterising the spatial dynamics of sympatric *Aedes aegypti* and *Aedes albopictus* populations in the Philippines

Jennifer Duncombe[1], Fe Espino[2], Kristian Marollano[3], Aldwin Velazco[2], Scott A. Ritchie[4], Wenbiao Hu[1], Philip Weinstein[5], Archie C. A. Clements[1]

[1]*Infectious Disease Epidemiology Unit, School of Population Health, University of Queensland, Herston, Australia;* [2]*Parasitology Department, Research Institute for Tropical Medicine, Muntinlupa City, Metro Manila, Philippines;* [3]*Medical Entomology Department, Research Institute for Tropical Medicine, Muntinlupa City, Metro Manila Philippines;* [4]*School of Public Health, Tropical Medicine and Rehabilitation Sciences, James Cook University, Cairns, Australia;* [5]*Barbara Hardy Institute, University of South Australia, Adelaide, Australia*

Abstract. Entomological surveillance and control are essential to the management of dengue fever (DF). Hence, understanding the spatial and temporal patterns of DF vectors, *Aedes (Stegomyia) aegypti* (L.) and *Ae. (Stegomyia) albopictus* (Skuse), is paramount. In the Philippines, resources are limited and entomological surveillance and control are generally commenced during epidemics, when transmission is difficult to control. Recent improvements in spatial epidemiological tools and methods offer opportunities to explore more efficient DF surveillance and control solutions: however, there are few examples in the literature from resource-poor settings. The objectives of this study were to: (i) explore spatial patterns of *Aedes* populations and (ii) predict areas of high and low vector density to inform DF control in San Jose village, Muntinlupa city, Philippines. Fortnightly, adult female *Aedes* mosquitoes were collected from 50 double-sticky ovitraps (SOs) located in San Jose village for the period June-November 2011. Spatial clustering analysis was performed to identify high and low density clusters of *Ae. aegypti* and *Ae. albopictus* mosquitoes. Spatial autocorrelation was assessed by examination of semivariograms, and ordinary kriging was undertaken to create a smoothed surface of predicted vector density in the study area. Our results show that both *Ae. aegypti* and *Ae. albopictus* were present in San Jose village during the study period. However, one *Aedes* species was dominant in a given geographic area at a time, suggesting differing habitat preferences and interspecies competition between vectors. Density maps provide information to direct entomological control activities and advocate the development of geographically enhanced surveillance and control systems to improve DF management in the Philippines.

Keywords: dengue, *Aedes*, surveillance, control, Philippines.

Introduction

Dengue fever (DF) is the principal arbovirus in the Philippines. In 2010, reported DF incidence ranged from 36 cases per 100,000 population in the southern Autonomous Region in Muslim Mindanao to 372 cases per 100,000 population in the northern Cordillera Administrative Region (Duncombe et al., 2012). DF is endemic in the Philippines and epidemics occur perennially, generally during the wet season (June-November) when environmental conditions are ideal for the proliferation of the DF vectors *Aedes (Stegomyia) aegypti* (L.) and *Ae. (Stegomyia) albopictus* (Skuse).

Corresponding author:
Jennifer Duncombe
Infectious Disease Epidemiology Unit
Level 4, Public Health Building, School of Population Health
The University of Queensland, Herston, QLD 4006 Australia
Tel. +61 40 406 6753
E-mail: j.duncombe@uq.edu.au

The introduction of *Ae. aegypti* to Asia in the 19th century led to this species becoming the dominant DF vector in cities, in which it was better adapted than native *Ae. albopictus* (Lounibos, 2002; Gratz, 2004). Since then, *Ae. albopictus* has adjusted to urban environments, though it still favours areas of dense vegetation, and the two species reside sympatrically throughout Asia. Although *Ae. albopictus* is arguably the better larval competitor, *Ae. aegypti* is considered the more competent DF transmitter, perhaps due to its anthropophilic behaviour, and thus, is acknowledged as the primary DF vector (Gubler and Kuno, 1997).

Evidence for *Ae. albopictus* being a less efficient vector of DF has led to hypotheses about the public health benefits of *Ae. albopictus* replacing *Ae. aegypti* populations throughout the world (Lambrechts et al., 2010). However, *Ae. albopictus* should not be dismissed as a mere pest: it is a competent viral transmitter and has been shown to be more tolerant of cold conditions than *Ae. aegypti*; overwintering in areas with minimum temperatures below 0 °C (Lounibos, 2002). Therefore, in the near future we can expect that *Ae. albopictus* – and ultimately DF – will expand to areas previously uninhabited by vector competent *Aedes* mosquitoes, and the classic DF transmission season seen in some cooler tropical countries may be extended. As a result, greater morbidity and mortality associated with DF and other viruses transmitted by *Ae. albopictus*, such as chikungunya, is likely (Powers and Logue, 2007). Further, increasing co-circulation of DF virus serotypes in human populations with specific herd immunity may increase the incidence of dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), which are more severe forms of DF resulting from secondary infection with a different serotype (WHO, 2012).

The development of a publicly available vaccine is underway. In the meantime, DF management relies on entomological surveillance and control (Ooi et al., 2006; Morrison et al., 2008). Since the decentralization of the Philippines health system in the 1990s, local authorities have assumed responsibility for health system delivery and financing (Bossert and Beauvais, 2002). Consequently, DF management, including entomological surveillance and control, depends on local-level support and varies considerably between localities within the country (van den Berg et al., 2012). Because vector control strategies are time-consuming and expensive, they are generally only undertaken during epidemics and may include: physical removal of breeding sites, indoor residual spraying (IRS), outdoor spraying (fogging), and community education.

As geographical information systems (GIS) technology and geostatistical methods progress and become more accessible, knowledge of the spatial dynamics of *Aedes* populations increases and new opportunities to explore integrated surveillance and control solutions for DF-affected countries are presented (Eisen and Lozano-Fuentes, 2009; Higa, 2011). Commonly, GIS are used for visualising the spread and density of vector breeding sites, determining *Aedes* distribution patterns and targeting areas for control (Sithiprasasna et al., 2004; Moreno-Sanchez et al., 2006; Tsuda et al., 2006; Lozano-Fuentes et al., 2008; Chang et al., 2009). More innovative techniques include predicting high-risk transmission zones (Carbajo et al., 2001; Ali et al., 2003; Getis et al., 2003; Chansang and Kittayapong, 2007). A number of advanced spatial and spatio-temporal analyses of DF cases in the Asia-Pacific region have also been undertaken (Hu et al., 2009, 2012; Li et al., 2012). However, there are few examples of applying spatial epidemiological tools to examine the dynamics of local sympatric *Aedes* populations and to develop targeted species-specific approaches to vector control in resource-limited settings.

This study characterises spatial patterns of *Ae. aegypti* and *Ae. albopictus* in San Jose village, Muntinlupa city in the Philippines. Specifically, the aim was to explore coexistence patterns and spatial clusters of *Aedes* populations and to predict vector density across the study area in order to identify high and low risk transmission zones at a fine spatial scale.

Materials and methods

Study area and data collection

Fortnightly, *Aedes* surveillance data (numbers of adult female *Ae. aegypti* and *Ae. albopictus* mosquitoes captured) were obtained from 50 double-sticky ovitraps (SOs) in San Jose village, Muntinlupa city for the period June-November 2011 (Fig. 1). San Jose village is a gated community of approximately 200 households located 30 km south of Manila (Duncombe and Marollano, 2011). The environment includes areas of dense bush land and has low population density (2.48 per km^2). Of the 50 SOs, 48 were placed under cover outside private homes and two were situated in public spaces near a community café. Households were selected using a spatial grid sampling design to facilitate geospatial analysis. Final selection of households was determined based on: (i) consent to participate by householders; (ii) accessibility of the property; and (iii) an adequate level of personal safety for fieldworkers.

SOs, designed to attract gravid adult female *Aedes* mosquitoes, are valuable tools for entomological surveillance because they are relatively inexpensive, portable and do not require electricity (Ritchie et al., 2004; Morrison et al., 2008; Chadee and Ritchie, 2010). They each comprise a top bucket lined with a polybutylene adhesive panel and a base bucket filled with water containing 0.5 g of organic attractant (i.e. lucerne) and a pellet of methoprene, which is an insect growth regulator that prevents mosquito maturation (Ritchie and Russell, 2002; Ritchie et al., 2003). These SOs were used throughout the work presented here with the base bucket cleaned and the water and pellets replenished each week.

During fieldwork, *Aedes* mosquitoes were extracted from the adhesive panel of each SO, stored in individual plastic vials (pooled by SO) and transported to the Medical Entomology Laboratory at the Research Institute of Tropical Medicine (RITM), Muntinlupa,

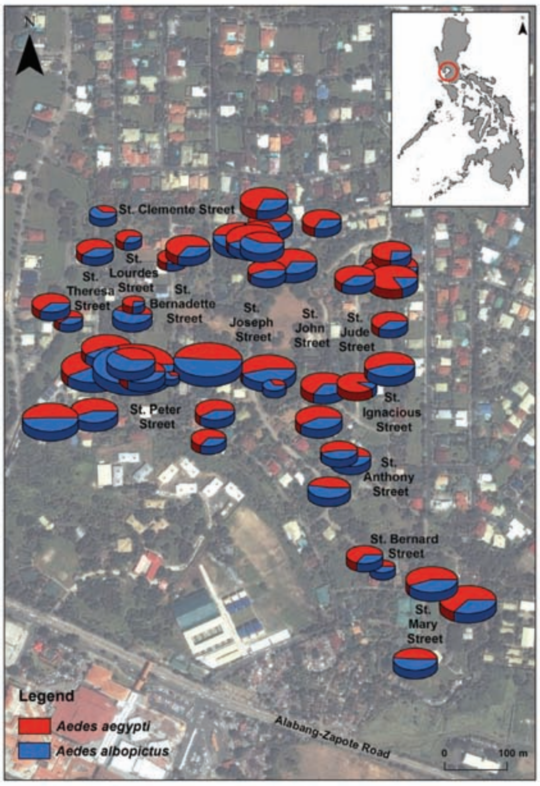

Fig. 1. Average fortnightly numbers of *Ae. aegypti* and *Ae. albopictus* for the study period (June-November 2011), sized according to average number of mosquitoes per sticky ovitrap (SO). See insert for the location of the study area in the Philippines.

the Philippines. Here, the species and sex of each mosquito was confirmed by Rueda's reference key via a Leica EZ4 stereo microscope (Rueda, 2004); approximately 5% of mosquitoes were damaged in or on removal from SOs and were visually identified in the field for species and sex.

One household (SO no. 18) was sent a letter (in English and Tagalog) in September following consistently high numbers of *Aedes* mosquitoes collected in this particular SO, and reports of suspected DF cases in the village. The letter informed the householders of the high numbers of *Aedes* found in the SO and suggested cleaning roof gutters and covering a nearby drain to remove possible breeding sites and reduce the likelihood of DF infection. The householders followed these recommendations and the number of *Aedes* collected in the SO decreased in the following weeks. Another household (SO no. 33) commenced indoor and outdoor insecticide spraying twice weekly in September 2011, subsequent to the hospitalization of multiple household members with suspected DF. Both of these incidents may have affected the number of adult female *Aedes* mosquitoes identified in SOs.

Spatial cluster analysis

Preliminary associations between fortnightly numbers of adult female *Ae. aegypti* and *Ae. albopictus* mosquitoes were visualised via mapping in the GIS software ArcMap, version 10.0 (ESRI; Redlands, USA).

The detection of spatial clustering within the study area during the study period was performed using the spatial scan statistic assuming a retrospective Poisson probability model in the SaTScan software, version 8.0 (Kulldorff, 1997). The spatial scan statistic centres a circular, isotropic window on each of the possible centroids throughout the geographical area, calculating the observed and expected number of mosquitoes in each circle and identifying the most likely and secondary (high or low) clusters. Markov chain Monte Carlo (MCMC) simulations (n = 999) were conducted under the assumption of spatial randomness to determine the statistical significance of the identified clusters. The case data used for the spatial Poisson model was the sum of the mosquitoes for the study period by SO for a given species. The population was the total number of *Aedes* mosquitoes by SO for the study period. To avoid duplication of results (i.e. testing different sides of the same ratio) only one species was required for testing of cases; we selected *Ae. aegypti*. The maximum spatial cluster size was equal to 50% of the population at risk and the statistical significance of the clusters was specified at P = 0.05. Due to the relatively short study period, spatio-temporal analysis was not performed.

Spatial interpolation

Spatial autocorrelation was assessed by generating exponential semivariograms using the *variog* function in the *GeoR* package of R statistical software (R Development Core Team, 2012). Semiovariograms were created by species for each of the 10 time periods (fortnights), as well as for the total study period. Data comprised the total number of adult female *Ae. aegypti* and *Ae. albopictus* at each of the 50 SO georeferenced locations for each time period. Covariance parameters were estimated by fitting weighted least squares (WLS) models to the semivariograms using the *variofit* function in the *GeoR* package.

Spatial interpolation methods are valuable for data where the variable of interest (e.g. mosquito density) is spatially continuous but is only measured at selected sites (e.g. in SOs). Kriging (Matheron, 1963) is a robust spatial interpolation technique that creates a prediction surface based on measured values (e.g. mosquito density in SOs), distance to prediction locations and a mathematical model of spatial autocorrelation informed by semivariogram covariance parameters (Pfeiffer et al., 2008). Ordinary kriging was selected as the most appropriate kriging model because it assumes an unknown mean which is defined by nearby data values; and thus, is more dynamic than other kriging models, such as simple kriging, which uses a pre-defined known mean for all locations.

For time periods in which spatial autocorrelation was detected using the semivariograms, a grid of 4,900 prediction locations (70 x 70 m) covering the study area was created. Each grid location was rectangular because of the shape of the study site and measured 12 by 17 m. Ordinary kriging was performed on the grid using the *pois.krige* and *pois.glm.control* functions in the *GeoRglm* package of R, and *Aedes* density estimates (counts) were obtained for each prediction location. The MCMC Metropolis-Hastings algorithm based on Langevin-Hastings updates and parameterisation specified in semivariography was employed to simulate the conditional distributions for gridded locations given data from SOs (Papaspiliopoulus et al., 2003). The number of MCMC iterations performed was 1,000, thinning was specified as 1 and burn-in set to 0. The proposal variance was scaled to a value between 0.5 and 0.7, assessed by trial and error for each time period, to ensure that approximately 60% of the proposals were accepted as required by Langevin-Hastings updates (Christensen and Waagepetersen, 2002). Further details of ordinary kriging using R are available elsewhere (Christensen and Ribeiro, 2012). The gridded kriging estimates were exported to ArcMap using raster image files created in the R *rgdal* package.

Results

The spatial distribution of *Ae. aegypti* and *Ae. albopictus*, and average number of mosquitoes at each SO location in the study area, is presented in Fig. 1. The temporal pattern of *Aedes* populations through the study period showed that *Ae. aegypti* mosquitoes are dominant in the first half of the study period only (Fig. 2). *Ae. aegypti* mosquito numbers increased from June to July and rapidly declined towards August. They increased again from August to September, though not as markedly and then declines towards November. Conversely, *Ae. albopictus* numbers declined from June to July, increased to a peak in September and decreased again towards November.

Spatio-temporal population dynamics are explored for *Aedes* mosquitoes in Figs. 3 and 4. As expected, *Ae. aegypti* specimens were more common in the residential areas in the northeast, while *Ae. albopictus* was dominant in the southwest where there is more vegetation and fewer houses. The maps show opposing areas of high vector density suggesting that, despite *Ae. aegypti* and *Ae. albopictus* residing sympatrically in San Jose village, only one species dominates each geographical area. These patterns were confirmed by spatial cluster detection, with a significantly low primary cluster of *Ae. aegypti* in the west (relative risk (RR) = 0.60, P = 0.002) and a significantly high, larger secondary cluster of *Ae. aegypti* in the east (RR = 1.25, P = 0.002) (Fig. 5).

Spatial autocorrelation was apparent in *Ae. aegypti* populations for the following periods: June 14-November 2 (whole study period); June 14-28; July 26-August 9; and October 4-18 (Fig. 6). Spatial autocorrelation was evident for *Ae. albopictus* mosquitoes for the period October 4-18 only. Semivariograms

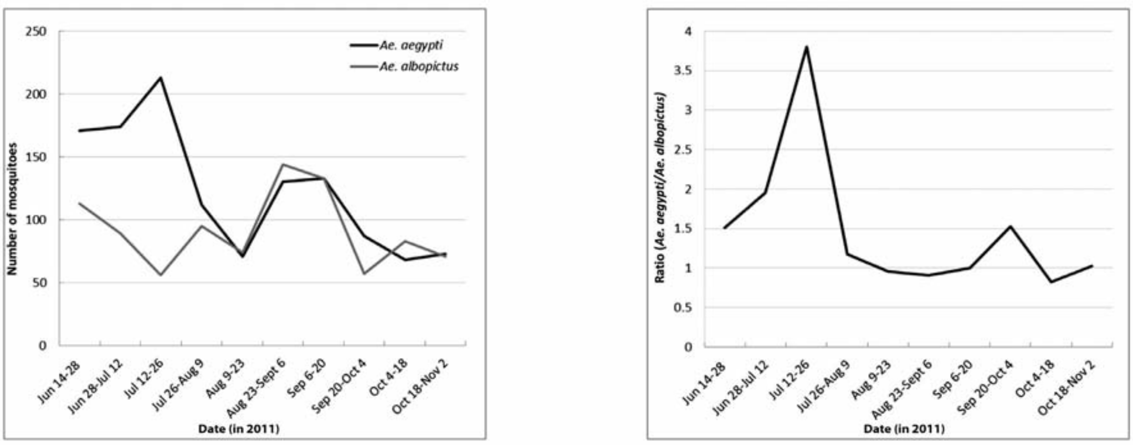

Fig. 2. The total number and ratio of *Ae. aegypti* and *Ae. Albopictus* mosquitoes by fortnight for the study period.

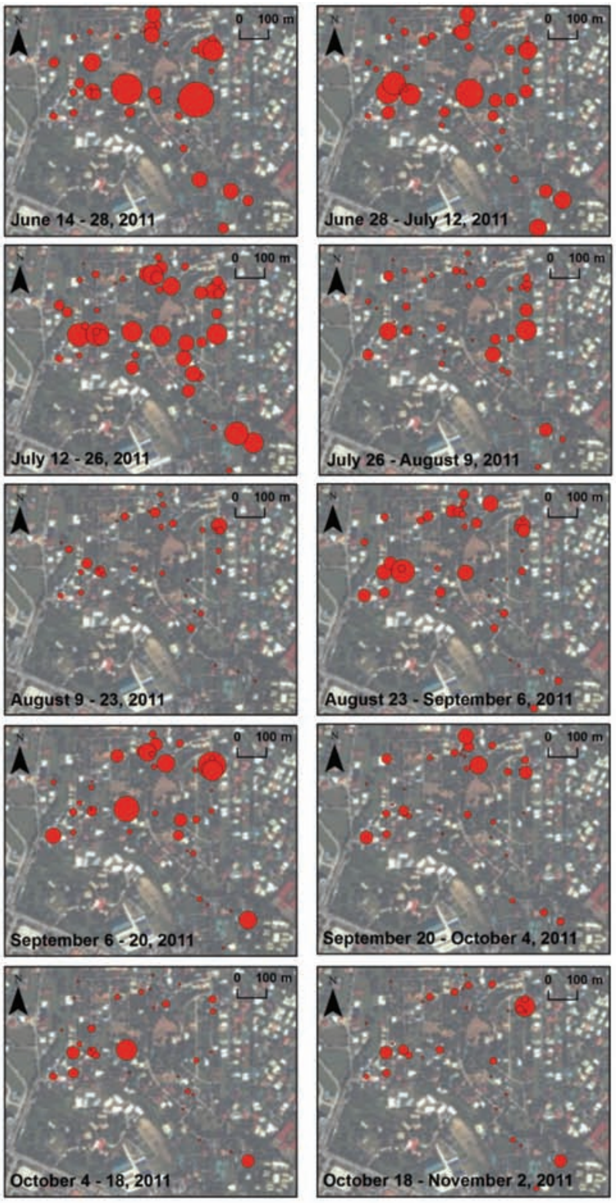

Fig. 3. Total fortnightly numbers of *Ae. aegypti* by time period, sized according to the total number of mosquitoes per sticky ovitrap (OS).

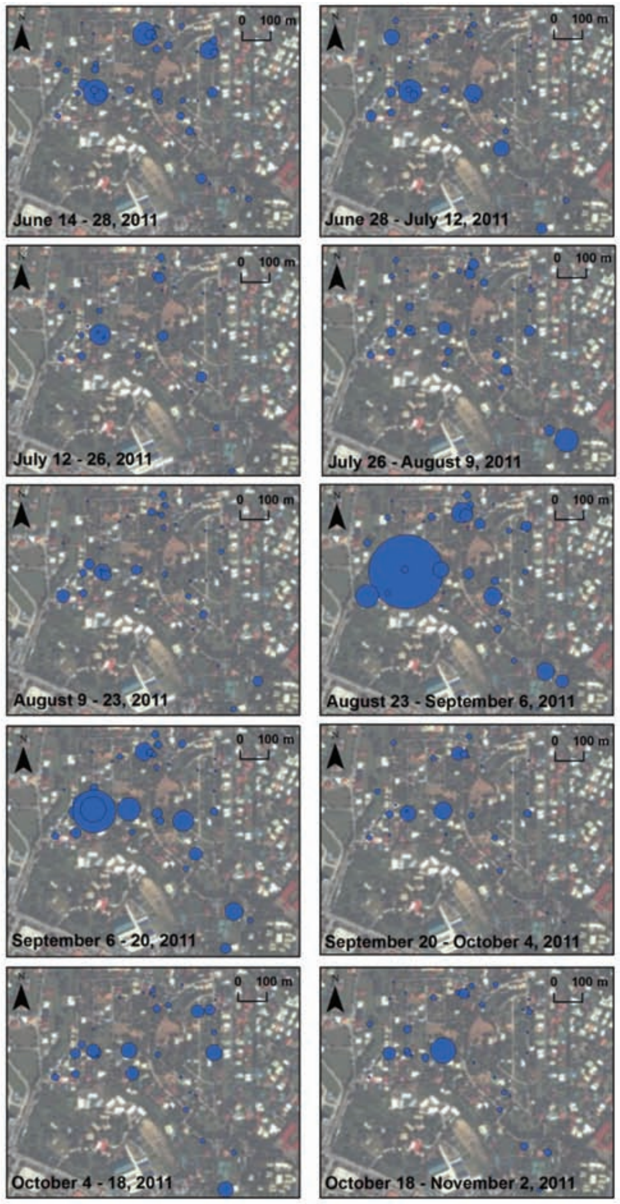

Fig. 4. Total fortnightly numbers of *Ae. albopictus* by time period, sized according to the total number of mosquitoes per sticky ovitrap (OS).

indicate spatial autocorrelation up to a distance of 100 m; that is, the number of *Aedes* mosquitoes at a given SO location was autocorrelated with the number of mosquitoes at other SOs located within 100 m.

Fig. 7 presents kriging maps of interpolated densities for *Ae. aegypti* and *Ae. albopictus* in the study site for the given time periods. Kriging estimates for *Ae. aegypti* populations reveal similar patterns through time periods with high density in the east-northeast of the study area, except for October 4-18 where density was highest in the west. For the same period, kriging estimates for *Ae. albopictus* demonstrated high density in the south of the study area.

Discussion

Our results differ from findings from a recent multi-site study undertaken in Southeast Asian countries, where *Ae. albopictus* was not identified in the Philippines or neighbouring countries (Arunachalam et al., 2010). Additionally, a larval and pupal survey undertaken in Muntinlupa city in 2008 identified *Ae. aegypti* but not *Ae. albopictus* mosquitoes (Cruz et al., 2008). We demonstrate here coexistence of *Ae. aegypti* and *Ae. albopictus* in San Jose village in the Philippines; which is consistent with a 25-year-old study undertaken in cemeteries in Manila, finding the

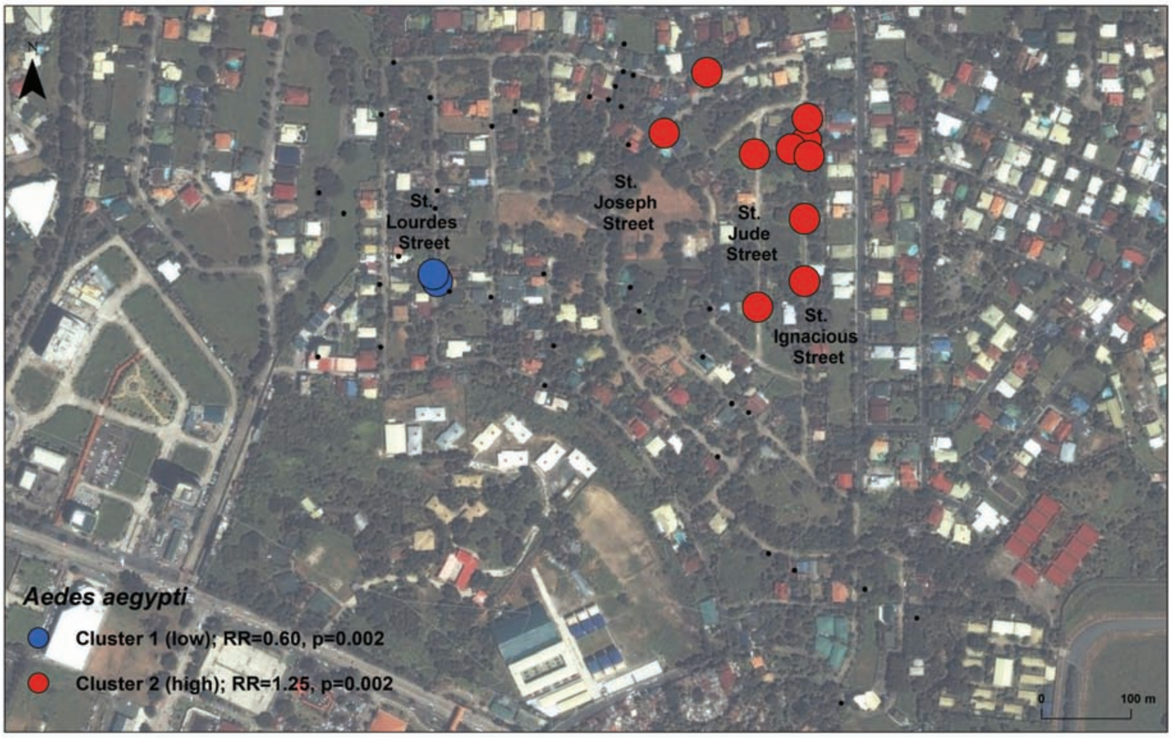

Fig. 5. Spatial cluster detection for total numbers of *Ae. aegypti* and *Ae. albopictus* mosquitoes for the study period.

presence of both species and showing that *Ae. albopictus* dominate vegetated areas (Schultz, 1989, 1993). More recent entomological surveillance, undertaken in a semi-rural area of the Philippine island of Palawan, reveals comparable rates of *Ae. aegypti* and *Ae. albopictus,* confirming our findings (unpublished data). A number of other countries in Asia, including Singapore, Thailand, Malaysia, and Vietnam, have also reported *Aedes* coexistence (Chan et al., 1971; Yap, 1975; Yap and Thiruvengadam, 1979; Kay et al., 2002).

To our knowledge, this is the first study to use robust geostatistical methods to explore sympatric *Ae. aegypti* and *Ae. albopictus* populations. We found that, although *Aedes* mosquitoes reside sympatrically, they prefer different locations in the study area. Generally, where density of *Ae. aegypti* was found to be high, that of *Ae. albopictus* was low, and *vice versa*. This is likely due to preferences for different environments (residential *versus* rural) and interspecies competition resulting in one species dominating a given geographical area, as shown by Juliano et al. (2004) and Tsuda et al. (2006). Indeed, increased numbers of *Ae. albopictus* mosquitoes in vegetative areas later in the wet season may extend spatial and temporal opportunities for DF transmission, which would not be possible if *Ae. aegypti* were the sole vector. Exploration of sympatric *Aedes* populations is important because evidence suggests that greater competition resulting from coexistence of species may result in increases in vector competence (Alto et al., 2008). Physiological characteristics of adult mosquitoes, including body size and wing length may be influenced by competition and other ecological interactions in the larval stage and may, therefore, affect adult vector competence for pathogens such as DF. However, most of the evidence for interspecies competition comes from laboratory-based studies that do not provide information intended for the control of diseases such as DF in real-world settings.

There was no observed spatial autocorrelation in the majority of time periods throughout the study, possibly due to small numbers in the dataset for those time periods. Spatial autocorrelation for *Ae. aegypti* in June 14-November 2, June 14-28, July 26-August 9 and October 4-18, and for *Ae. albopictus* during October 4-18, was found up to a distance of 0.001 decimal degrees (approximately 100 m). This information is potentially useful for vector surveillance because it indicates that SOs could be placed approximately 100 m apart to provide comprehensive surveillance coverage, i.e. closer placement would waste surveillance resources.

Interpolated vector density maps showed similar high vector density zones throughout the study period for *Ae. aegypti* populations in the east of the study

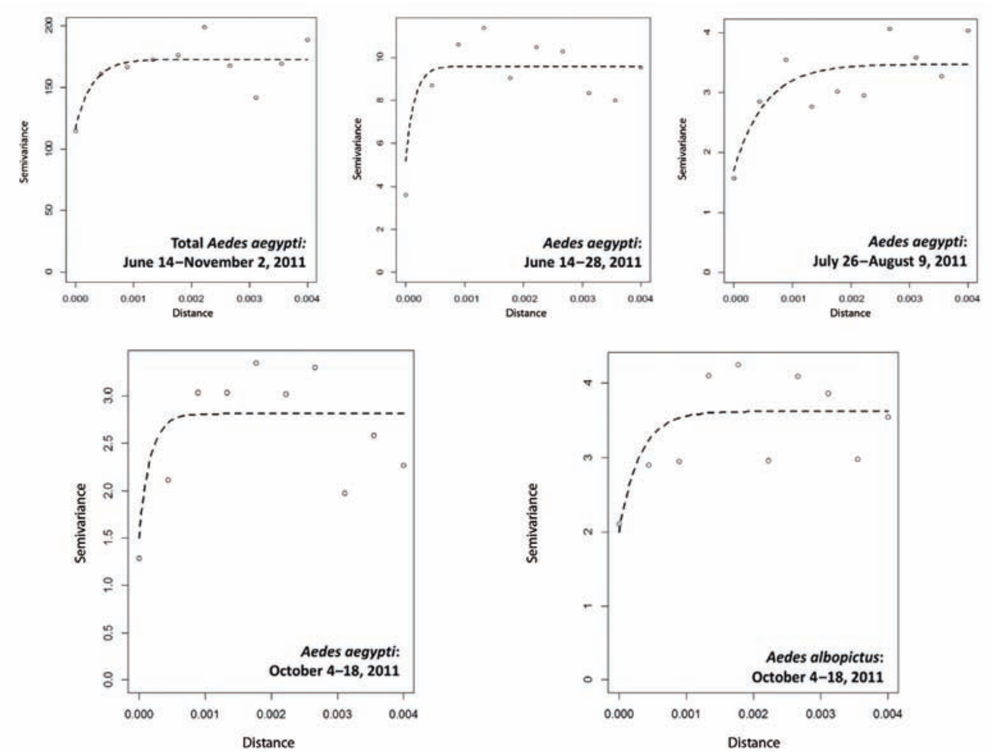

Fig. 6. Semivariograms demonstrating spatial autocorrelation, by time period and species, up to a lag distance of 0.001 decimal degrees (equivalent to approximately 100 m at the Equator).

area. However, high vector density for *Ae. aegypti* during October 4-18 was seen in the west and *Ae. albopictus* density was highest in the south of the study area. Spatial differences in species preferred locations may be influenced by habitat characteristics previously mentioned (e.g. residential *versus* vegetative location), host location and oviposition behaviour. Indeed, *Ae. aegypti* are more anthropophilic than *Ae. albopictus*, which might also blood feed on vertebrates other than humans, and thus are more likely to oviposit inside homes or in close proximity to humans (Barrera, 1996). Mosquito density maps showing high transmission zones can be used to advocate for better resources and improved targeting of prevention activities. Evidence supports prevention and control programmes at the household level or container level for maximum protection (Arunachalam et al., 2010). Maps may be used to identify areas of low transmission risk so that valuable resources may be oriented away from these areas and better utilised in other, high-risk, areas.

The development of integrated, sub-national surveillance systems, such as spatial decision support systems (SDSS), could assist the planning, targeting and implementation of prevention and control programmes (Kelly et al., 2010, 2011, 2012; Eisen and Eisen, 2011). In particular, SDSS could improve the speed, accuracy and efficiency of control activities during an epidemic, such as IRS, where maintaining a high spraying coverage rate is essential for ensuring control effectiveness and limiting DF transmission (Vazquez-Prokopec et al., 2010). SDSS may also determine risk areas for DHF and DSS epidemics following the introduction of a new virus serotype in a population with herd immunity to previous virus serotypes. Additionally, SDSS may improve the coordination of control activities between localities, facilitate resource allocation decisions *via* user-defined reports and enable data collection and reporting standardisation across large countries like the Philippines.

The assessment of some vector control strategies has been undertaken in the Philippines, including the use of the microbial agent *Bacillus thuringiensis israelensis* in water containers (de Melo-Santos et al., 2001; Mahilum et al., 2005), and insecticide-treated curtains inside houses (Madarieta et al., 1999). A multifaceted vector control approach, that includes environmental management and chemical and biological methods, has also been suggested (Su, 2008). In 2011, the Department of Science and Technology (DOST) commenced a multimillion-peso project to distribute ovicidal/larvicidal (OL) traps to schools and households nationwide with the aim of reducing the number of female *Aedes* mosquitoes and therefore interrupting

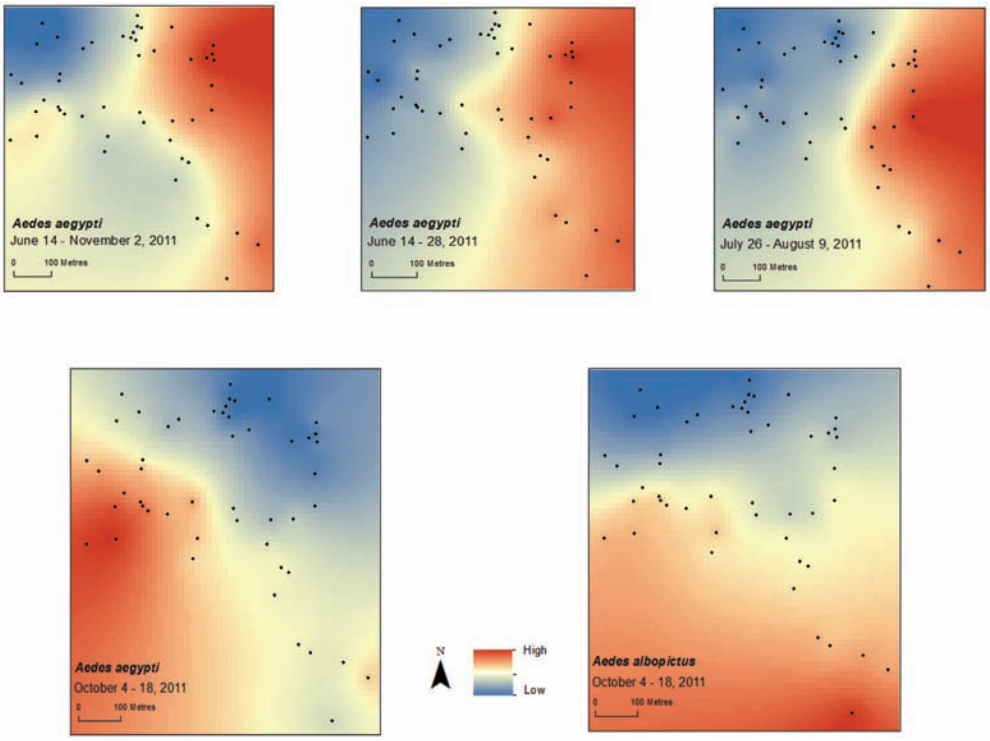

Fig. 7. Ordinary kriging estimates by species for each time point demonstrating spatial autocorrelation for June 14-28, July 26-August 9 and October 4-18, as well as for the whole study period (June 14-November 2).

DF transmission. Unfortunately, deficient field testing of OL traps prior to commencement and a lack of community education on the use and maintenance of the traps (potentially transforming them into *Aedes* breeding sites) may limit the effectiveness of the project (Briones et al., 2012). Other concerns include ongoing maintenance costs of OL traps for schools and households, and the availability of local health staff to assist the placement of OL traps, therefore, reducing their capacity to perform other duties.

Neighbouring countries have also tried a number of vector control strategies for DF prevention. In Cambodia, for example, larvicidal and insecticidal controls have been tested, including the use of larvivorous fish (*Poecilia reticulate*) in rural communities to control *Ae. aegypti* in water storage containers (Seng et al., 2006, 2008a, 2008b, 2008c; Suaya et al., 2007). In Vietnam, the crustacean *Mesocyclops* (Copepoda) has been utilised in a preventative vector control programme based on community-mobilization (Kay et al., 2002; Kay and Vu, 2005). The bacterium *Wolbachia* – used to shorten the lifespan of *Ae. aegypti* mosquitoes – is currently being tested in Vietnam (Jeffery et al., 2009; Hurst et al., 2012). A more comprehensive, but resource-intensive, vector control programme was implemented in the Plaeng Yao district, Thailand, employing community education, physical removal of oviposition sites, lethal ovitraps, biological controls and insecticides (Kittayapong et al., 2006). Further research in this area should focus on establishing sustainable vector control programmes employing community mobilization and considering local ecological, biological and social factors (Heintze et al., 2007; Arunachalam et al., 2010; van den Berg et al., 2012). Additionally, the response of *Ae. albopictus* to vector control strategies should be investigated following difficulties implementing control programmes in other parts of the world (Paupy et al., 2009).

The current study has some limitations, including the short study period (5 months). A longer time series is required to establish seasonal patterns of *Aedes* mosquitoes and determine the feasibility of using SOs in areas with different socio-demographic and environmental characteristics. Additionally, the spatial sampling frame was compromised because of concerns with property accessibility and personal safety. Another limitation was the lack of funding available for virologic surveillance, such as reverse transcription-polymerase chain reaction (RT-PCR) assays, which can be undertaken on field-collected *Aedes* mosquitoes to determine virus infection rates and serotypes by species, and may act as an early warning system for epidemics (Chow et al., 1998). However, mosquitoes have been stored appropriately for use if funding becomes available. Although spatial analysis was performed based on a latitude and longitude geographical coordinate system, which may have impacted distance measures provided, the study area was close to the Equator, and thus, the results were likely unaffected.

Our results show that *Aedes* mosquitoes cohabitate in San Jose village but have opposing spatial clustering patterns, suggesting interspecies competition and differing habitat preferences. Mosquito density maps identify potential high and low risk DF transmission zones for directing prevention and control activities, and may be used to advocate the development of targeted local-level surveillance such as SDSS for integrated DF management in the Philippines. We recommend further studies to confirm our findings and determine DF infection rates and seroprevalence rates in *Aedes* field-collected mosquitoes to provide evidence for the association between vector density and DF transmission. Applied research identifying, implementing and evaluating sustainable community-based vector control programmes should also be prioritised. Additionally, confirmation of the vector competence of local *Ae. albopictus* populations is required to determine whether ongoing habitat expansion in locations with sympatric *Aedes* populations would increase the global health threat from DF.

Ethical approvals

Human research ethics applications submitted to the RITM Institutional Review Board (2011-10) and the University of Queensland Medical Research Ethics Committee (2011000490) were approved.

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