Updated abundance and distribution of *Aedes albopictus* (Skuse) (Diptera: Culicidae) in Penang Island, Malaysia

Rahim, J.¹, Ahmad, A.H.^{1*}, Maimusa, A.H.¹ and Irfan Shah²

¹School of Biological Sciences, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia ²University of Agriculture Faisalabad (UAF) Sub-Campus, Toba Tek Singh, Pakistan

*Corresponding author e-mail: stegoculex@gmail.com

Received 6 July 2017; received in revised form 21 January 2018; accepted 22 January 2018

Abstract. Reduction of dengue cases and forecast its risk, and identification of vectors breeding habitats and their abundance is the prime target in any dengue control programme. In this aspect, larval surveys were conducted in four localities in Penang Island between September 2015 to September 2016. The abundance of Aedes mosquitoes and their breeding habitat both indoor and outdoor were recorded. Aedes indices i.e. Container index (CI), House index (HI) and Breteau Index (BI) were calculated for dengue risk, besides the attraction and repulsion (RF) of 5 container type evaluation. Among a total of 2,415 potential habitats examined, 638 were found positive for immature stages of Aedes. A total of 23,319 immatures were collected from the selected areas. Aedes albopictus (93.7%) was the dominant species followed by Aedes aegypti (5.8%) and others (0.5%). Among the 5 container types, plastic type containers were the most productive (45.5%) whereas the natural containers (6.1%) were the least (P < 0.05). High values of Aedes indices showed that all selected localities are at risk of dengue due to high prevalence of Ae. albopictus. Rubber and natural type of containers were the most attractive breeding habitats for vectors of dengue. The results of this study provides an insight to the current distribution of dengue vectors, which may be crucial to the health authorities in vector management programmes in the future.

INTRODUCTION

Some genera of the Culicidae family, namely Anopheles, Aedes and Culex are competent vectors of the several diseases and have greatly contributed to the spread of these deadly pathogens around the globe. These three genera are the primary interest of entomologists due to their special role in the outbreaks of malaria, dengue, Zika and chikungunya (Weaver & Reisen, 2010). In 2014, the World Health Organization (WHO) estimated that malaria and dengue contribute up to 17.0% of the global burden of infectious diseases (WHO, 2014). Recent studies have shown a decline in malaria, and an upsurge in dengue and zika incidences (Benelli & Mehlhorn, 2016). Approximately half of the world population is in danger of dengue which is evident from the increased number

of dengue cases in recent years (Bhatt *et al.*, 2013). The upsurge in the number of dengue cases is due to the global expansion of these two highly competent vector species, namely *Aedes aegypti* and *Aedes albopictus* (Kraemer *et al.*, 2015). In Malaysia, dengue was first reported in Penang in 1902 (Skae, 1902) and remained endemic in the country with 223 reported mortalities in 2016 (WHO, 2017).

Aedes aegypti is highly anthropohilic and prefers to reside inside or near human dwellings in the urban areas (Juliano & Lounibos, 2005). Whereas *Ae. albopictus* is an aggressive, exophagic and exophilic mosquito, which can be found in rural and suburban areas and usually breeds in outdoor environment (Paupy *et al.*, 2009). However, the overlapping distribution of both container dwelling vectors has been documented from Malaysia (Chen *et al.*, 2006; Rozilawati *et al.*, 2007; Roslan *et al.*, 2013).

In Malaysia, Ae. albopictus is considered the most important vector species due to its epidemiological capability as a carrier of several arboviruses. This species, especially in Penang Island, is the most relevant vector species which has drawn the attention of the vector control authorities. Because of its adaptation to indoor breeding behavior (Dieng et al., 2010), along with its abundance in the urban, suburban (Rozilawati et al., 2007), and rural areas (Saifur et al., 2012a). Ae. albopictus has also been documented as one of the dominant species in dengue outbreak areas (Mohiddin et al., 2015; Rozilawati et al., 2015) as well as in communal spots (Maimusa et al., 2017). Being a superior competitor at the larval stage in nature, and capable of displacing other Aedes species, it has been found in major proportion as compared to other Ae. species (Braks et al., 2004; Kesavaraju et al., 2014). Similar observations have been reported during the larval surveillance study in the urban areas of Penang Island, where Ae. albopictus was found replacing Ae. aegypti (Maimusa et al., 2017).

Regardless of several novel advances in dengue virus and vector control efforts and their implementations, Malaysia has attained a limited success in dengue control (Lee et al., 2015). Although, several vector control strategies have been integrated to control these mosquitoes, breeding source destruction is still the key practice to maintain vector population below a threshold level (Bowman et al., 2016). Prior to the source destruction activities, identification of the potential breeding habitats of dengue vectors is essential for its effectiveness (Saifur et al., 2013), which eventually reduces the use of insecticides. Adequate information on vector abundance and spatial distribution of a species is considered as a critical factor to predict the risk of diseases caused by mosquito vectors (Juliano & Lounibos, 2005; Kweka et al., 2015). It is urged to periodically monitor vector density in a disease control or vector surveillance program (Britch et al., 2008). In addition, the identification and

productivity of the most relevant containers is required (Valenca *et al.*, 2013).

Human activities like urbanization, deforestation and the use of insecticides have altered the behavior of dengue vectors. These changes in breeding habitats have already been reported in both species in Penang Island. Ae. aegypti is found breeding outdoor (Saifur et al., 2012b), and Ae. albopictus has successfully adapted to indoor breeding environment (Dieng et al., 2010). Familiarities with such changes in the breeding ecology of these vectors are crucial for the management of control interventions. A few vector surveillance studies, using ovitrap and identification of breeding habitats have been carried out in Penang Island between the period of 2009 to 2011 (Saifur et al., 2012a; Saifur et al., 2013; Mohiddin et al., 2015; Rozilawati et al., 2015). However, to the best of our knowledge no further study has been conducted on the breeding habitats of Aedes around human dwellings since then.

This study, therefore seeks to fulfil the gaps and to update the current status of vector abundance and preferred breeding habitats in Penang Island using larval survey method.

MATERIALS AND METHODS

The present study was undertaken in four residential areas of tropical Penang Island, Malaysia over a period of 13-months. The Island is located between latitudes 5° 8_N and 5° 35_N and longitudes 100° 8_E and 100° 32_E. The climate is tropical, with a temperature range of 23.5 to 31.4°C, relative humidity (RH) of 60.9 to 96.8%, and average annual rainfall range from 2670 to 3250 mm (Ali et al., 2011). However, during the study period the monthly temperature was between 26.2 to 29.0°C, with a RH of 69.8 to 82.8% and average rainfall of 30.2 to 507.4 mm was reported. The metrological data was obtained from the Penang meteorological station, Bayan Lepas Pulau Pinang, Malaysia. This study was undertaken in four residential areas of Penang namely, Gelugor, Sungai Nibong (Sg. Nibong), Permatang Damar Laut (P.D. Laut), and Balik Pulau. Container surveys were initiated in September 2015 and completed in September 2016.

On fort-nightly basis, selected localities were surveyed entomologically, starting from 0900 to 1500 h, with 2 teams of 3 persons constituted with at least one medical entomologist. Before visiting a house, permission was acquired from the house member to survey the premises and the purpose of study was clarified using Bahasa Melayu (Malay) translated letter of introduction as described previously (Rahim et al., 2016). A very keen and thorough observations of the houses were attempted. All the accessible houses were inspected for the indoor and outdoor breeding habitats of Aedes, spending 10-15 minutes per house. Here we referred indoor containers that were under the house structure (porches), however, those outside the structure but within the territory of the houses were classified as outdoor containers as mentioned elsewhere (Rozilawati et al., 2015). The containers were considered as positive when found infested even with a single immature. Besides this, natural breeding sites other than the artificial containers like tree holes and some plants axils were also inspected. All containers, containing water, were examined for the presence of immature and the size and holding capacity of containers were recorded. The aquatic content containing immature were poured into plastic bags, and labelled according to the date, site and type of the containers. Depending on the size and situation of breeding source, larvae and pupae were collected using turkey baster, small fishing net or with small plastic pipette from small breeding places. Torch light was used to observe the presence of larvae in the dark conditions. The content of turbid containers was sieved and shifted into plastic containers, containing clean water to observe the presence of immatures. All preimaginal stages were transported to the laboratory on the same day. Maximum individuals were identified at larval stage, whereas the remaining were cultured to adults and identified using the keys provided by (Pratt, 1969; Rattanarithikul et al., 2010).

Data analysis

Containers were categorized into 3 sizes such as; containers with water holding capacity of > 10 liter were considered as large, > 1liter and < 10 were medium and containers with holding capacity of <1 Liter were classified as small. Meanwhile, positive containers were identified under 5 different categories by type namely: plastic, metal, cement, rubber and natural. Entomological indices, the Container index (CI) (percentage of positive containers with immatures), the House index (HI) (percentage of infested houses) and the Breteau index (BI) (number of infested containers per 100 houses surveyed) for each of the locality were calculated to determine the Aedes larval densities. The values of CI > 3, HI > 4 and BI> 5 were taken as the indicators of dengue epidemic risk (Weeraratne et al., 2013). Risk factor (RF) for the 5 container types (X) was calculated using the following formula.

No. of infested containers X / no. of infested containers
$$RF_X =$$

No. of potential breeding habitats X / no. of potential breeding habitats

Equivalent to:

$$RF_X = \frac{CI_X}{CI}$$

The values of RF greater than 1 indicates the attractiveness of the containers (at risk), whereas a value less than 1 means the containers are not attractive (no risk) (Favier *et al.*, 2006; Weeraratne *et al.*, 2013). After the data were tested for normality, differences between the containers size and number of immature were analyzed using One-way ANOVA, while distribution of *Aedes* immature among the localities was compared using the non-parametric Kruskal-Wallis test. All the data were subjected to SPSS version 22 for the analysis and the significant differences were expressed at P value < 0.05.

RESULTS

A total of 800 houses were visited during the survey period. Among the visited houses 186 (23.3%) were found positive for *Aedes*, of which a higher percentage of positive houses was reported from P.D. Laut (28.5%), followed by Balik Pulau (27.0%), Sg. Nibong (21.5%) and the Gelugor (16.0%). Among these *Aedes* infected houses, 49 (26.3%) houses were found with indoor breeding whereas 137 (73.7%) houses with outdoor breeding (Table 1). Among the indoor positive houses, 20.4% were positive with *Ae. aegypti* while 79.6% of houses were positive with *Ae. albopictus*. The percentage of indoor and outdoor positive houses for both species are presented by locality in Table 1.

In all the locations, a total of 2,415 potential breeding habitats were found and out of these habitats 638 were found positive with *Aedes* immatures. Among the positive habitats, a total of 23,319 immatures were found. *Aedes albopictus* (93.7%) was the dominant species in all the locations followed by *Ae. aegypti* (5.8%). In addition to both *Aedes* species, *Culex* and *Toxorhynchites* (0.5%) immatures were also observed.

Statistical analysis, Kruskal Walis Test, showed that the distribution of immature was similar among the localities ($\chi^2 = 1.467$, df = 3, P > 0.05) (Table 2). Besides mosquito larvae, an uncounted number of red midge larvae (Chironomids) was observed in the containers.

Table 3 shows that in all the surveyed localities, plastic type containers contained the highest number of immatures with a positive percentage of 45.2%, followed by metal (22.8%), cement (17.1%), rubber (8.8%), and natural (6.1%) containers. Significant differences were seen between the containers types in terms of immature productivity ($\chi^2 = 16.614$, df = 4, P < 0.05). Whereas, medium size containers were the most imperative to produce huge number of immatures with a percentage positivity of 45.3% followed by large and small size containers with values of 26.6 and 27.1%, respectively. One-way ANOVA test results reveals that there is no difference between

Location	Total houses visited	Indoor positive	Outdoor positive	Total (%)	Indoor positive houses Ae. aegypti	Indoor positive houses Ae. albopictus	Outdoor positive Ae. aegypti	Outdoor positive houses Ae. albopictus
Gelugor	200	9	23	32 (16.0%)	03	6	6	17
Sg. Nibong	200	11	32	43 (21.5%)	06	5	4	28
P.D. Laut	200	14	43	57 (28.5%)	01	13	06	37
Balik Pulau	200	15	39	54 (27.0%)	0	15	3	36
Total	800	49	137	186 (32.2%)	10	39	19	118

Table 1. Total number of houses positive indoor and outdoor in selected localities with both species

Total 2. Total number of positive containers and immature collected from the four locations during the study period

Locations	Total positive containers	Ae. albopictus	Ae. aegypti	Others (Culex + Toxorhynchites)	Total	
Gelugor	126	3,217	669	49	3,935	
Sg. Nibong	133	5,186	393	33	5,612	
P.D.Laut	197	7,042	187	20	7,249	
Balik Pulau	182	6,401	109	13	6,523	
Total	638	21,846 (93.7%)	1,358 (5.8%)	115 (0.5%)	23,319	

Kruskal Wallis test P > 0.05.

Containers						
type	Gelugor Total (%)	Sg. Nibong Total (%)	P.D. Laut Total (%)	Balik Pulau Total (%)	Total (%)	
Plastic	1,791 (45.5%)	2,862 (51.0)	3,434 (47.4)	2,449 (37.5)	10,536 (45.2)	
Metal	980 (24.9)	1,122 (20.0)	1,763 (24.3)	1,459 (22.4)	5,324 (22.8)	
Cement	568 (14.4)	730 (13.0)	1,162 (16.0)	1,527 (23.4)	3,987 (17.1)	
Rubber	305 (7.8)	561 (10.0)	548 (7.6)	630 (9.7)	2,044 (8.8)	
Natural	291 (7.4)	337 (6.0)	342 (4.7)	458 (7.0)	1,428 (6.1)	

Table 3. Total number of immatures and productivity¹ of containers (%) by five types in four localities

 1 Productivity = number of immatures collected \times 100/ total number of immatures. Kruskal Wallis test P < 0.05.

Table 4. Total number and percentage of immatures collected from four localities by size

Container size					
	Gelugor Total (%)	Sg. Nibong Total (%)	P.D. Laut Total (%)	Balik Pulau Total (%)	Total (%)*
Large	773 (19.6)	1,627 (29.0)	1,958 (27.0)	2,087 (32.0)	6,445 (27.6)
Medium	2,061 (52.4)	2,246 (40.0)	3,769 (52.0)	2,480 (38.0)	10,556 (45.3)
Small	1,011 (28.0)	1,739 (31.0)	1,522 (21.0)	1,956 (30.0)	6,318 (27.1)

* One way ANOVA values showed that no significant difference in the number of immatures P = 0.056.

Table 5. Total and individual *Aedes* indices Container index (CI), House index (HI) and Breteau (BI) for *Ae. albopictus* and *Ae. aegypti* calculated for the four study sites

		¹ Gelugor		Sg. Nibong		P.D. Laut			Balik Pulau			
	² AL	$^{3}\!AG$	Total	^{2}AL	$^{3}\!AG$	Total	^{2}AL	$^{3}\!AG$	Total	^{2}AL	$^{3}\!AG$	Total
CI	18.9	2.8	20.9	24.5	1.4	25.9	26.6	2.3	28.9	28.8	0.6	29.5
HI	14	4.5	18.5	19.5	2	21.5	25	3.5	28.5	26	1	27
BI	57	8.5	63	63	3.5	66.5	90.5	8	98.5	89	2	91

¹Mixed breeding only in 5 containers.

²AL, Ae. albopictus; ³AG, Ae. aegypti.

the number of immatures for all size container (F = 4.037, df = 2, P = 0.056) (Table 4).

Table 5 shows the values of *Aedes* indices for both species in the four selected locations. During the survey period, mixed breeding of both *Aedes* species was observed only in Gelugor in 5 containers. Values of *Aedes* indices higher than the defined values (CI > 3, HI > 4 and BI > 5) showed that all the localities are at risk of dengue. The HI individual indices value of 4.5 for *Ae. aegypti* shows that Gelugor is at moderate risk, but on the hand, Gelugor and P.D. Laut are at risk due to high BI values of 8.5 and 8, respectively due to this species. Whereas, all the localities are at high risk of dengue due to the massive existence of *Ae. albopictus. Aedes* indices values showed that Balik Pulau has a high epidemic risk, whereas Gelugor has the least. Mosquito's immatures were found almost all year round. The highest CI, HI and BI were recorded in September 2016, whereas the lowest values were recorded in March 2016. Overall monthly *Aedes* indices are presented in Figure 1.



Figure 1. Monthly *Aedes* indices house index (HI), Breteau index (BI), and container index (CI) in selected localities during the study period.



Figure 2. Relationship between rainfall and number of immatures in four selected localities.

A significant positive correlation was found between the rainfall and the number of immatures at all locations. At Gelugor, the relationship for rainfall was r = 0.925, P < 0.001, Sg. Nibong (r = 0.860, P = < 0.001), Balik Pulau (r = 0.732, P = 0.004), whereas a weak relationship was observed in P.D. Laut between rainfall and number of immatures (r = 0.617, P = 0.025) (Figure 2). The correlation of temperature with number of immatures at Gelugor was insignificant (r = 0.029, P > 0.05), Sg. Nibong (r = 0.003, P > 0.05), P.D. Laut (r = 0.004, P > 0.05) and Balik Pulau (r = 0.003, P > 0.05). Similarly, no significant correlation was found between the number of immatures and relative

humidity in all the locations i.e. Gelugor was (r = 0.002, P > 0.05), Sg. Nibong (r = 0.106, P > 0.05), P.D. Laut (r = 0.142, P > 0.05), Balik Pulau (r = 0.156, P > 0.05).

Among the positive breeding container types, rubber type containers, tyres and shoes were the most attractive breeding habitats in all the localities. Plastic and cement containers were the second most attractive breeding habitats. Metal and cement type containers were the repulsive habitats with the marginal risk factor value of 0.9 in Gelugor. In Sg. Nibong the high RF value (2) was observed for rubber type breeding habitats, followed by natural, cement, metal and the least was plastic with the values of 2.0, 1.6, 1.3, 1.0 and 0.9, respectively. While in P.D. Laut, except natural containers (RF = 0.8), the rest of container types were attractive with the range of RF values 1.0 to 1.5. Metal type containers were the repulsive (RF = 0.8)breeding habitats in Balik Pulau while the attractive values of 1.9, 1.7, 1.3 and 1.2 for rubber, cement, natural and plastic type containers respectively were observed.

DISCUSSION

Malaysia is a endemic country for dengue due to its favorable climatic conditions for the successful breeding of mosquitoes. Aedes indices obtained from immature inspections in dengue outbreak areas have been the motive for the vector surveillance program in Malaysia (Azil et al., 2011). Results of the present study showed that Ae. albopictus is the dominant species in the selected localities. During 2009, a vector surveillance study conducted in Penang Island revealed that Ae. aegypti was the dominant species in urban areas of Penang, including Gelugor and Ae. albopictus was the dominant species in highly vegetative (Rural) areas (Saifur et al., 2012a). However, the results presented here reveals that Ae. albopictus (82.2%) has currently become the dominant species in the urban areas of Gelugor as compared to Ae. aegypti (17.2 %). These findings supported the evidences of recent field studies on public places which showed that it was

dominated by *Ae. albopictus* in Penang Island (Maimusa *et al.*, 2017). Chen *et al.* (2009) reported the complete absence of *Ae. aegypti* in a suburban areas of Kuala Lumpur. In a separate study conducted in the highly developed area of Shah Alam (Malaysia), *Ae. albopictus* (90.7%) was found to be the dominant species (Faiz *et al.*, 2017).

The findings of dengue vector surveillance studies in Malaysia are consistent with studies carried out in different countries, showing a major decline in the abundance of Ae. aegypti and the invasion of Ae. albopictus in the urban areas (Hobbs et al., 1991; Kaplan et al., 2010; Beilhe et al., 2012; Weeraratne et al., 2013). As reported, Aedes albopictus is an aggressive mosquito species which competes with other species in several ways and it is has the competitive advantages over Ae. aegypti in field conditions (Juliano et al., 2004; Juliano, 2010). Inter-specific competition between Aedes species have reported in several studies which showed the the displacement of Ae. aegypti (Lounibos, 2002) and Ae. sierrensis (Kesavaraju et al., 2014) by Ae. albopictus in their native areas. Although, the current results do not provide a competition between these two species, but the low density of Ae. aegypti provides evidence that Ae. albopictus has invaded the urban areas of Penang.

The present results indicate that Ae. aegypti is not completely displaced from the containers and possibly may have adopted different breeding habitats in Penang Island in a similar way as reported in Brazil (Paploski et al., 2016) and Singapore (Seidahmed & Eltahir, 2016). In these two countries, the storm drains were found containing Ae. aegypti population. Moreover, other environmental factors such as, high temperature and precipitation could be the reasons for the successful growth of Ae. albopictus (Neto & Navarro-Silva, 2004) as compared to the development of Ae. aegypti (Grech et al., 2015). The temperature can reach up to 35°C in Penang Island (Saifur et al., 2013), and this temperature range is unfavorable for the pupation of Ae. aegypti (Kumar et al., 2016).

The ovitrap and larval surveys conducted on Penang Island during 2011 by Rozilawati et al. (2015) reported high abundance of Ae. albopictus in ovitraps as compared to Ae. aegypti and vice versa for containers in the selected localities of Gelugor and P.D. Laut. The present larval study reveals that Ae. albopictus was dominant in containers in both studied areas. Meanwhile, a low percentages of Ae. aegypti larvae encountered during the survey confirmed its presences in Balik Pulau. Previous findings by Saifur et al. (2012a) reported this area was free of Ae. aegypti between 2009–2010 and predicted that the frequent traffic from urban areas may cause this species to spread to this area.

Several characteristics of plastic such as, durability, easy to recycle, light weight, and low cost, has encouraged the industries to design plastic made products for a wide range of usage. Together with several socioeconomic factors, plastic type containers have been found the most productive breeding habitats in several parts of the world (Chareonviriyaphap et al., 2003; Banerjee et al., 2015; Verna, 2015; Dhar-Chowdhury et al., 2016), including Malaysia (Saifur et al., 2013; Rozilawati et al., 2015; Faiz et al., 2017; Maimusa et al., 2017). During the present study period, a wide range of plastic type containers were found dispersed and suitable for Ae. albopictus breeding.

Routine inspection of Aedes infested containers is a standard method in vector control program (Chadee, 2004). The values of Aedes indices (CI > 3, BI > 4 and HI > 5) observed in the current study indicates that, the population density of Aedes and the potential breeding habitats were high enough to be a threat for dengue outbreaks. Results of the present larval survey showed that all the selected localities on Penang Island are at dengue risk due to the high prevalence of Ae. albopictus. Previously, this vector species was also found dominant in an ovitrap based study conducted in dengue outbreak areas of Penang Island (Mohiddin et al., 2015). Aedes albopictus is a competent vector for all dengue serotypes, and naturally contain dengue virus during the dry season (Thenmozhi et al., 2007). In Malaysia,

transovarial transmission of serotype 2 has been detected from the wild caught larvae of *Ae. albopictus* (Rohani *et al.*, 2014).

Regarding the indoor and outdoor breeding preference of both vectors, the observations were similar to the previously conducted studies on Penang Island (Saifur et al., 2012a; Rozilawati et al., 2015). Both indoor and outdoor containers were found feasible for Ae. albopictus breeding. However, high number of positive containers was seen outside the houses, which serves as more potential breeding habitats during the rainy season as compared to indoor containers. It is likely due to the high nutrient contents and the lesser frequency of changing water in outdoor containers as compared to indoor containers. In an early investigation, similar probabilities were also observed for Ae. *aegupti* breeding in outdoor containers in Iquitos, Peru (Schneider et al., 2004). In general, female Ae. albopictus preferably lays eggs in more than 2 containers during a single gonotrophic cycle (Davis et al., 2015; Davis et al., 2016). Moreover, skip oviposition behavior may also assist Ae. albopictus in harboring more outdoor containers.

Interestingly, high number of Ae. aegupti immature was collected after the moderate rain fall as compared to low rain fall season lasted in the month of April 2016 starting from January (Same year). A possible reason for such observation can be the egg desiccation tolerance of both Aedes species. As compared to Ae. albopictus, eggs of Ae. aegypti are more resistant to the drought environmental conditions (Juliano et al., 2002), which favors Ae. aegypti by reversing competitive advantages (Juliano & Lounibos, 2005). Furthermore during the dry season in Bangladesh, Chowdhury et al. (2014) observed Ae. albopictus females utilizing treeholes debris for the survival of their further progenies.

Undouble, the direct impact of climatic conditions such as rain fall and temperature pose great impact on the life aspects of mosquitoes. As stated before, several studies have found that dengue vector and disease cases are positively associated with environmental conditions. Besides the positive impact of rain fall, simulated field trials have shown a negative impact of heavy rain fall on immatures (Dieng et al., 2012). A significant impact of with rain fall, while an insignificant association between temperature relative humidity and the number of immatures was noted in this study. An increase of 0.5–1.5°C in the temperature of Malaysia have been observed between years 1998–2007, whereas 27°C is considered as an average temperature reported by Hii et al. (2016). In an earlier research, Saifur et al. (2013) reported an up surge in the temperature up to 35°C in Penang during their survey in 2009. Besides the effect of temperature range on vector biology, it also has an impact on the dengue virus replication and incubation period.

Along with the other characteristics, attraction in Aedes towards black colour is their innate behaviour and a huge number of studies have reported that types are the most preferred breeding habitat of these vector species. Laboratory based studies on the oviposition behaviour of Ae. albopictus have also shown the preferred attraction towards black colour jars (Yap et al., 1995). In this study, high RF was observed for rubber and natural containers compared to other artificial habitats. In another study, the CI values for natural containers (44.4%) and (37.9%) for tires have been reported from Shah Alam (Faiz et al., 2017). The attraction towards colour and high nutrients in natural containers makes them attractive for the oviposition. Plastic type containers accounted for 45.0% of all immature collected and was highly abundant but do not posed a high risk factor. In Sri Lanka, similar patterns of RF were found for both dengue vectors for tyres, however the leaf axils were repulsive breeding habitats (RF=0.64) for Ae. albopictus (Weeraratne et al., 2013). In a separate study, Morrison et al. (2004) stated that, targeting plastic containers in a source destruction programme is inappropriate due to its high abundance but low infestation rate of 3.6% as compared to the other containers.

Current survey shows that, *Ae. albopictus* is the main competing vector in Penang Island. However, further studies are required for deeper understandings of how larval competition and type of containers can affect

the life history traits and abundance of this vector species. Beside this, the influence of environmental variables, quality and quantity of resources in containers on adults should be studied. The interspecific mating studies between Ae. albopictus and Ae. aegypti should be carried out in field to investigate the distribution of species. Furthermore, vectorial capacity of these vectors should be studied in the areas where they coexist. Several vector surveillance studies have been carried out on Penang Island, but still there is no account of circulating virus type in these species. Besides targeting key breeding containers, it is also essential to survey and target storm drains and drainage lines which facilitate the breeding of Ae. *aegypti* in some areas.

Acknowledgments. The authors would like to thank the Administration of School of Biological Sciences for providing services during the study period. Author Junaid Rahim would also like to thank TWAS – USM postgraduate Fellowship programme (FR# 3240275093) for pursuing this study at USM. This research was funded by Long Term Research Grant (LRGS) for Infectious Diseases Ministry of Higher Education Malaysia (USM-304/PBIOLOGI/650575/ U112).

REFERENCES

- Ali, M., Ahmad, F., Yahaya, A. & Farooqi, M. (2011). Characterization and hazard study of two areas of Penang Island, Malaysia. *Human and Ecological Risk* Assessment 17: 915-922.
- Azil, A.H., Li, M. & Williams, C.R. (2011). Dengue vector surveillance programs: a review of methodological diversity in some endemic and epidemic countries. *Asia-Pacific Journal of Public Health* 23: 827-842.
- Banerjee, S., Aditya, G. & Saha, G.K. (2015). Household Wastes as Larval Habitats of Dengue Vectors: Comparison between Urban and Rural Areas of Kolkata, India. *PLoS ONE* 10: e0138082.

- Beilhe, L.B., Arnoux, S., Delatte, H., Lajoie, G. & Fontenille, D. (2012). Spread of invasive Aedes albopictus and decline of resident Aedes aegypti in urban areas of Mayotte 2007–2010. Biological Invasions 14: 1623-1633.
- Benelli, G. & Mehlhorn, H. (2016). Declining malaria, rising of dengue and Zika virus: insights for mosquito vector control. *Parasitology Research* **115**: 1747-54.
- Bhatt, S., Gething, P.W., Brady, O.J., Messina, J.P., Farlow, A.W., Moyes, C.L., Drake, J.M., Brownstein, J.S., Hoen, A.G. & Sankoh, O. (2013). The global distribution and burden of dengue. *Nature* **496**: 504-507.
- Bowman, L.R., Donegan, S. & McCall, P.J. (2016). Is Dengue Vector Control Deficient in Effectiveness or Evidence?: Systematic Review and Meta-analysis. *PLoS Neglected Tropical Diseases* 10: e0004551.
- Braks, M.A.H., Honório, N., Lounibos, L., Lourenço-de-Oliveira, R. & Juliano, S. (2004). Interspecific competition between two invasive species of container mosquitoes, *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae), in Brazil. *Annals of the Entomological Society of America* 97: 130-139.
- Britch, S.C., Linthicum, K.J., Anyamba, A., Tucker, C.J. & Pak, E.W. (2008). Longterm surveillance data and patterns of invasion by *Aedes albopictus* in Florida. *Journal of the American Mosquito Control Association* 24: 115-120.
- Chadee, D.D. (2004). Key premises, a guide to *Aedes aegypti* (Diptera: Culicidae) surveillance and control. *Bulletin of Entomological Research* **94**: 201-207.
- Chareonviriyaphap, T., Akratanakul, P., Nettanomsak, S. & Huntamai, S. (2003). Larval habitats and distribution patterns of *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse), in Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health* **34**: 529-535.
- Chen, C.D., Lee, H.L., Stella-Wong, S.P., Lau, K.W. & Sofian-Azirun, M. (2009). Container survey of mosquito breeding sites in a university campus in Kuala Lumpur, Malaysia. *Dengue Bulletin* **33**: 187-193.

- Chen, C.D., Nazni, W.A., Lee, H.L., Seleena, B., Mohd Masri, S., Chiang, Y.F. & Sofian-Azirun, M. (2006). Mixed breeding of *Aedes aegypti* (L.) and *Aedes albopictus* Skuse in four dengue endemic areas in Kuala Lumpur and Selangor, Malaysia. *Tropical Biomedicine* 23: 224-227.
- Chowdhury, R., Chowdhury, V., Faria, S., Huda, M.M., Laila, R., Dhar, I., Maheswary, N.P. & Dash, A.P. (2014). How dengue vector *Aedes albopictus* (Diptera: Culicidae) survive during the dry season in Dhaka City, Bangladesh? *Journal of Vector Borne Diseases* 51: 179-187.
- Davis, T.J., Kaufman, P.E., Hogsette, J.A. & Kline, D.L. (2015). The Effects of Larval Habitat Quality on Aedes albopictus Skip Oviposition. Journal of the American Mosquito Control Association **31**: 321-328.
- Davis, T.J., Kaufman, P.E., Tatem, A.J., Hogsette, J.A. & Kline, D.L. (2016).
 Development and Evaluation of an Attractive Self-Marking Ovitrap to Measure Dispersal and Determine Skip Oviposition in *Aedes albopictus* (Diptera: Culicidae) Field Populations. *Journal of Medical Entomology* 53: 31-38.
- Dhar-Chowdhury, P., Haque, C.E., Lindsay, R. & Hossain, S. (2016). Socioeconomic and Ecological Factors Influencing *Aedes aegypti* Prevalence, Abundance, and Distribution in Dhaka, Bangladesh. *American Journal of Tropical Medicine and Hygiene* **94**: 1223-1233.
- Dieng, H., Rahman, G.M., Abu Hassan, A., Che Salmah, M.R., Satho, T., Miake, F., Boots, M. & Sazaly, A. (2012). The effects of simulated rainfall on immature population dynamics of *Aedes albopictus* and female oviposition. *International Journal of Biometeorology* **56**: 113-120.
- Dieng, H., Saifur, R.G., Ahmad, A.H., Salmah, M.R., Boots, M., Satho, T., Jaal, Z. & AbuBakar, S. (2010). Indoor-breeding of *Aedes albopictus* in northern peninsular Malaysia and its potential epidemiological implications. *PLoS ONE* 5: e11790.

- Faiz, M., Nazri, C.D. & Chua, S.T. (2017). Spatial and Temporal distribution of *Aedes* (Diptera: Culicidae) mosquitoes in Shah Alam. *Tropical Biomedicine* 34: 1-9.
- Favier, C., Degallier, N., Vilarinhos Pde, T., de Carvalho Mdo, S., Yoshizawa, M.A. & Knox, M.B. (2006). Effects of climate and different management strategies on Aedes aegypti breeding sites: a longitudinal survey in Brasilia (DF, Brazil). Tropical Medicine & International Health 11: 1104-1118.
- Grech, M.G., Sartor, P.D., Almiron, W.R. & Luduena-Almeida, F.F. (2015). Effect of temperature on life history traits during immature development of *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae) from Cordoba city, Argentina. *Acta Tropica* **146**: 1-6.
- Hii, Y.L., Zaki, R.A., Aghamohammadi, N. & Rocklov, J. (2016). Research on Climate and Dengue in Malaysia: A Systematic Review. Current Environmental Health Reports 3: 81-90.
- Hobbs, J.H., Hughes, E.A. & Eichold, B.H. (1991). Replacement of Aedes aegypti by Aedes albopictus in Mobile, Alabama. Journal of the American Mosquito Control Association 7: 488-489.
- Juliano, S.A. (2010). Coexistence, exclusion, or neutrality? A meta-analysis of competition between *Aedes albopictus* and resident mosquitoes. *Israel Journal* of Ecology & Evolution **56**: 325-351.
- Juliano, S.A. & Lounibos, L.P. (2005). Ecology of invasive mosquitoes: effects on resident species and on human health. *Ecology Letters* **8**: 558-574.
- Juliano, S.A., Lounibos, L.P. & O'Meara, G.F. (2004). A field test for competitive effects of *Aedes albopictus* on *A. aegypti* in South Florida: differences between sites of coexistence and exclusion? *Oecologia* **139**: 583-593.
- Juliano, S.A., O'Meara, G.F., Morrill, J.R. & Cutwa, M.M. (2002). Desiccation and thermal tolerance of eggs and the coexistence of competing mosquitoes. *Oecologia* **130**: 458-469.

- Kaplan, L., Kendell, D., Robertson, D., Livdahl, T. & Khatchikian, C. (2010). Aedes aegypti and Aedes albopictus in Bermuda: extinction, invasion, invasion and extinction. Biological Invasions 12: 3277-3288.
- Kesavaraju, B., Leisnham, P.T., Keane, S., Delisi, N. & Pozatti, R. (2014). Interspecific Competition between Aedes albopictus and A. sierrensis: potential for Competitive Displacement in the Western United States. PLoS ONE 9: e89698.
- Kraemer, M.U., Sinka, M.E., Duda, K.A., Mylne,
 A.Q., Shearer, F.M., Barker, C.M., Moore,
 C.G., Carvalho, R.G., Coelho, G.E., Van
 Bortel, W., Hendrickx, G., Schaffner, F.,
 Elyazar, I.R., Teng, H.J., Brady, O.J.,
 Messina, J.P., Pigott, D.M., Scott, T.W.,
 Smith, D.L., Wint, G.R., Golding, N. & Hay,
 S.I. (2015). The global distribution of
 the arbovirus vectors Aedes aegypti
 and Ae. albopictus. Elife 4: e08347.
- Kumar, G., Singh, R.K., Pande, V. & Dhiman, R.C. (2016). Impact of container material on the development of *Aedes aegypti* larvae at different temperatures. *Journal* of Vector Borne Diseases 53: 144-148.
- Kweka, E.J., Munga, S., Himeidan, Y., Githeko, A.K. & Yan, G. (2015). Assessment of mosquito larval productivity among different land use types for targeted malaria vector control in the western Kenya highlands. *Parasites & Vectors* 8: 1-8.
- Lee, H., Rohani, A., Khadri, M., Nazni, W., Rozilawati, H., Nurulhusna, A., Nor Afizah, A., Roziah, A., Rosilawati, R. & Teh, C. (2015). Dengue vector control in Malaysia – challenges and recent advances. *Internatonal Medical Journal* of Malaysia 14: 11-16.
- Lounibos, L.P. (2002). Invasions by insect vectors of human disease. *Annual Review of Entomology* **47**: 233-266.
- Maimusa, H.A., Ahmad, AH., Kassim, NFA., Ahmad, H., Dieng, H. & Rahim, J. (2017). Contribution of public places in proliferation of dengue vectors in Penang Island, Malaysia. Asian Pacific Journal of Tropical Biomedicine 7: 183-187.

- Mohiddin, A., Jaal, Z., Lasim, A.M., Dieng, H. & Zuharah, W.F. (2015). Assessing dengue outbreak areas using vector surveillance in north east district, Penang Island, Malaysia. *Asian Pacific Journal of Tropical Diseases* **5**: 869-876.
- Morrison, A.C., Gray, K., Getis, A., Astete, H., Sihuincha, M., Focks, D., Watts, D., Stancil, J.D., Olson, J.G., Blair, P. & Scott, T.W. (2004). Temporal and geographic patterns of *Aedes aegypti* (Diptera: Culicidae) production in Iquitos, Peru. *Journal of Medical Entomology* **41**: 1123-1142.
- Neto, P.L. & Navarro-Silva, M.A. (2004). Development, longevity, gonotrophic cycle and oviposition of *Aedes albopictus* Skuse (Diptera: Culicidae) under cyclic temperatures. *Neotropical Entomology* **33**: 29-33.
- Paploski, I.A., Rodrigues, M.S., Mugabe, V.A., Kikuti, M., Tavares, A.S., Reis, M.G., Kitron, U. & Ribeiro, G.S. (2016). Storm drains as larval development and adult resting sites for *Aedes aegypti* and *Aedes albopictus* in Salvador, Brazil. *Parasites* & Vectors **9**: 419.
- Paupy, C., Delatte, H., Bagny, L., Corbel, V. & Fontenille, D. (2009). *Aedes albopictus*, an arbovirus vector: from the darkness to the light. *Microbes and Infection* **11**: 1177-1185.
- Pratt, H.D. (1969). Workbook on the Identification of Mosquito Larvae. Atlanta, GA: US Department of Health, Education and Welfare.
- Rahim, J., Ahmad, A.H., Kassim, N.F.A., Ahmad, H., Ishak, I.H., Rusul, A.C. & Maimusa, H.A. (2016). Revised discriminating lethal doses for resistance monitoring programme on Aedes albopictus against temephos and malathion in Penang island, Malaysia. Journal of the American Mosquito Control Association **32**: 210-216.
- Rattanarithikul, R., Harbach, R.E., Harrison, B.A., Panthusiri, P., Coleman, R.E. & Richardson, J.H. (2010). Illustrated keys to the mosquitoes of Thailand. VI. Tribe Aedini. Southeast Asian Journal of Tropical Medicine and Public Health 41 Suppl 1: 1-225.

- Rohani, A., Azahary, A.A., Malinda, M., Zurainee, M., Rozilawati, H., Najdah, W.W. & Lee, H. (2014). Eco-virological survey of *Aedes* mosquito larvae in selected dengue outbreak areas in Malaysia. *Journal of Vector Borne Diseases* 51: 327-332.
- Roslan, M.A., Shafie, A., Ngui, R., Lim, Y.A. & Sulaiman, W.Y. (2013). Vertical infestation of the dengue vectors Aedes aegypti and Aedes albopictus in apartments in Kuala Lumpur, Malaysia. Journal of the American Mosquito Control Association 29: 328-36.
- Rozilawati, H., Tanaselvi, K., Nazni, W.A., Mohd Masri, S., Zairi, J., Adanan, C.R. & Lee, H.L. (2015). Surveillance of *Aedes albopictus* Skuse breeding preference in selected dengue outbreak localities, peninsular Malaysia. *Tropical Biomedicine* **32**: 49-64.
- Rozilawati, H., Zairi, J. & Adanan, C.R. (2007). Seasonal abundance of *Aedes albopictus* in selected urban and suburban areas in Penang, Malaysia. *Tropical Biomedicine* 24: 83-94.
- Saifur, R.G.M., Ahmad, A.H., Dieng, H., Ahmad, H., Salmah, M.R.C., Satho, T. & Saad, A.R., Morales Vargas, R.E. (2012a). Update on temporal and spatial abundance of dengue vectors in Penang, Malaysia. *Journal of the American Mosquito Control Association* 28: 84-92.
- Saifur, R.G.M., Dieng, H., Hassan, A.A., Salmah, M.R.C., Satho, T., Miake, F. & Hamdan, A. (2012b). Changing domesticity of *Aedes aegypti* in northern peninsular Malaysia: reproductive consequences and potential epidemiological implications. *PLoS ONE* 7: e30919.
- Saifur, R.G.M., Ahmad, A.H., Dieng, H., Salmah, M.R.C., Saad, A.R. & Satho, T. (2013). Temporal and spatial distribution of dengue vector mosquitoes and their habitat patterns in Penang Island, Malaysia. Journal of the American Mosquito Control Association 29: 33-43.
- Schneider, J.R., Morrison, A.C., Astete, H., Scott, T.W. & Wilson, M.L. (2004). Adult size and distribution of *Aedes aegypti* (Diptera: Culicidae) associated with

larval habitats in Iquitos, Peru. *Journal* of *Medical Entomology* **41**: 634-642.

- Seidahmed, O.M. & Eltahir, E.A. (2016). A Sequence of Flushing and Drying of Breeding Habitats of Aedes aegypti (L.) Prior to the Low Dengue Season in Singapore. PLoS Neglected Tropical Diseases 10: e0004842.
- Skae, F. (1902). Dengue fever in Penang. British Medical Journal 2: 1581-1582.
- Thenmozhi, V., Hiriyan, J.G., Tewari, S.C., Samuel, P.P., Paramasivan, R., Rajendran, R., Mani, T.R. & Tyagi, B.K. (2007).
 Natural vertical transmission of dengue virus in *Aedes albopictus* (Diptera: Culicidae) in Kerala, a southern Indian state. *Japanese Journal of Infectious Diseases* 60: 245-249.
- Valenca, M.A., Marteis, L.S., Steffler, L.M., Silva, A.M. & Santos, R.L. (2013). Dynamics and characterization of *Aedes aegypti* (L.) (Diptera: Culicidae) key breeding sites. *Neotropical Ento*mology 42: 311-6.
- Verna, T.N. (2015). Species Composition and Seasonal Distribution of Mosquito Larvae (Diptera: Culicidae) in Southern New Jersey, Burlington County. *Journal of Medical Entomology* 52: 1165-1169.
- Weaver, S.C. & Reisen, W.K. (2010). Present and future arboviral threats. *Antiviral Research* 85: 328-45.

- Weeraratne, T.C., Perera, M.D.B., Mansoor, M.M. & Karunaratne, S.P. (2013). Prevalence and breeding habitats of the dengue vectors *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in the semi-urban areas of two different climatic zones in Sri Lanka. *International Journal of Tropical Insect Science* 33: 216-226.
- W.H.O. (2014). A global brief on vector-borne diseases. [Internet]. Geneva, Switzerland:
 World Health Organization [accessed May 10, 2015]. Available from:http:// www.who.int/campaigns/world-healthday/2014/global-brief/en/.
- W.H.O. (2017). Update on the Dengue situation in the Western Pacific Region.
 [Internet]. Geneva, Switzerland: World Health Organization [assesed January 22, 2017]. Available from: http://www. wpro.who.int/emerging_diseases/ DengueSituationUpdates/en/.
- Yap, H.H., Lee, C.Y., Chong, N.L., Foo, A.E. & Lim, M.P. (1995). Oviposition site preference of *Aedes albopictus* in the laboratory. *Journal of the American Mosquito Control Association* 11: 128-132.