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Surveillance of *Aedes albopictus* Skuse breeding preference in selected dengue outbreak localities, peninsular Malaysia

Rozilawati, H.[1,2*], Tanaselvi, K.[1], Nazni, W.A.[1], Mohd Masri, S.[3], Zairi, J.[2], Adanan, C.R.[2] and Lee H.L.[1]
[1]Medical Entomology Unit, Institute for Medical Research, Jalan Pahang 50588 Kuala Lumpur, Malaysia
[2]Vector Control Research Unit, School of Biological Sciences, Universiti Sains Malaysia, 11 800 Pulau Pinang, Malaysia
[3]Strategic Resource Research Centre, Malaysian Agricultural Research Development Institute, PO Box 12301, 50744 Kuala Lumpur, Malaysia
[*]Corresponding author: rozilawati@imr.gov.my
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Abstract. Entomological surveillance was conducted in order to determine the abundance and to evaluate any changes of biological vectors or ecology, especially in the dengue outbreak areas. The abundance and breeding preference of *Aedes albopictus* and *Aedes aegypti* were conducted in selected dengue outbreak localities in three states of peninsular Malaysia namely Selangor, Federal Territory of Kuala Lumpur, and Penang Island using ovitraps and larval survey method. It was determined that *Ae. albopictus* was predominant in most of the localities and found to breed more outdoor than indoor. A wide range of breeding foci were recorded in this study. It was also determined that ovitrap method was more effective to detect the presence of *Aedes* mosquitoes when the larval survey was at low rate of infestation. The abundance of *Ae. albopictus* in dengue outbreak localities emphasis that the vector control programme should also target this species together with the primary dengue vector, *Ae. aegypti*.

INTRODUCTION

Entomological surveillance is often used to determine changes in the density and distribution of the vector to ensure timely response and control. Ovitrap and larval survey have been conducted in Malaysia to monitor the dengue vectors, mainly *Aedes aegypti* in urban and suburban areas.

Aedes albopictus Skuse has been known as an outdoor species that breeds in a broad range of natural environment, as well as artificial water holding containers (Hawley, 1988; Paupy *et al.*, 2009). It was known as a rural species (Hawley, 1988); however recently, it was reported that the species is adapted to urban and suburban areas in Malaysia, which overlaps with the distribution of *Ae. aegypti* with single or mixed infestation in the same breeding container (Yap, 1975; Chen *et al.*, 2006a; Rozilawati *et al*, 2007; Lau *et al.*, 2013). Even though it is known as an outdoor breeder, it was first reported during nationwide *Aedes* survey in peninsular Malaysia by Lee (1991) that the species could also be found indoor with *Ae. aegypti* but *Ae. albopictus* remained as the dominant outdoor breeder. Nevertheless, information on the abundance of this species in dengue outbreak areas in Malaysia is still limited. The wide distribution of *Ae. albopictus* in urban and suburban areas in Malaysia may indicate that the species can play an important role in transmitting dengue together with *Ae. aegypti*. The role of *Ae. albopictus* as dengue vector was highlighted with the absence of *Ae. aegypti* during series of outbreak in Japan, in the year 1942 which involved at least 200 000 cases (Gratz, 2004). Later it was also indicated that *Ae. albopictus* was the vector responsible for transmission of dengue in several other Asian region such as in India, Indonesia, Thailand and Malaysia (Birks, 1952; Reeves, 1959; Rudnick & Hammon, 1960; Surtees, 1970; Reuben *et al.*, 1988; Gratz, 2004).

Recently, Dieng *et al.* (2010) reported that the species was adapted to indoor breeding in several residential areas in Penang Island. This behaviour may impact on the control programme since it was targeted as an outdoor-breeding species. *Aedes albopictus* may adapt very well to human habitation and can be more abundant than *Ae. aegypti* in certain areas, and current dengue vector control approaches are more focused on the indoor breeding mosquitoes than the outdoor breeder, (Gratz, 2004).

Thus, the objective of this study was to determine the breeding preference and abundance of *Ae. albopictus* in selected dengue prone localities in Selangor, Federal Territory of Kuala Lumpur and Penang Island of peninsular Malaysia using the ovitrap and larval survey method.

MATERIALS AND METHODS

Study localities

Entomological investigations were conducted in dengue outbreak areas in Selangor, Federal Territory of Kuala Lumpur, and Penang Island. These three states were selected from the outbreak data obtained from Vector Borne Disease Control Programme, Ministry of Health, which showed the highest dengue cases in Malaysia between 2010 and 2011. The localities selected were classified into urban and suburban areas. A locality is classified as an outbreak area if a second case is reported within 14 days from the first reported case, whereas an uncontrollable outbreak is defined as the occurrence of new cases after 14 days from the second reported case. A locality is considered a hotspot when the duration of outbreak reaches 30 days and beyond the day the outbreak started. The survey began in January to June of 2011, with a gap of two weeks for each locality. The sites selected for this study are listed and described in Tables 1 indicating the GPS co-ordinates and the residential types involved.

Mosquito collection method

Two methods were used in this study: Oviposition trap (ovitrap) and larval surveys. In this study, both survey methods were performed indoor and outdoor. Indoor refers to those structures under a roof, while outdoor is those outside the roof but within the immediate vicinity of the premise.

Oviposition traps (ovitrap)

Using the guidelines provided by the Ministry of Health Malaysia (1997), a total of 60 ovitraps per locality were placed in 30 randomly selected houses/premises. An ovitrap is a black plastic container (base diameter 6.5 cm, opening diameter 7.8 cm, and height 9.0 cm). Twenty four hours before an ovitrap was introduced in the field, the ovitrap was filled with 225 mL dechlorinated tap water together with two surfaced hardboard paddles (size 10 cm X 2.5 cm X 0.3 cm), which were provided as the oviposition sites for *Aedes* females to oviposit.

One ovitrap was placed inside (indoor) a house/premise and one outside (outdoor) of the same house/premise. Before the ovitraps were placed in the premises, the owners were briefed on the purpose of placing the ovitrap inside and outside of their premises and informed consent was obtained. The ovitraps were placed continuously for 5 days in the field. All collected ovitraps were recorded on prescribed forms. Each ovitrap was closed with a lid and brought back to the laboratory The eggs were hatched to larval or adult stage and identified.

Ovitrap data analysis

i) The abundance of *Aedes* mosquito collected by species

ii) The ovitrap index (OI) was calculated as:

$$\text{Ovitrap Index (OI)} = \frac{\text{Number of positive ovitrap}}{\text{Number of ovitrap collected}} \times 100$$

Table 1. The study localities in Selangor, Kuala Lumpur and Penang Island

State	Locality	GPS Coordinate	Category	Houses/ residential type	Ecological condition
Selangor	Kg. Sg. Ramal Dalam, Kajang, Selangor	N 02° 58.039’ E 101° 45.456'	Suburban	Scattered Mostly single houses/ premises	Surrounded with various vegetation such as trees and shrubs. The environment is not properly maintained and managed.
	Intanaria Apartment, Bangi, Hulu Langat	N 02° 58.180’ E 101° 46.852'	Urban	Well planned Apartment	Have vegetation in the surrounding area. A well-managed and clean area.
	Selayang K4	N 03°14.954’ E 101° 40. 244'	Urban	Planned Single houses Terraces	Surrounded with vegetation, shrubs. The environment is generally not properly managed.
	Taman Paling Jaya	N 02°57.400’ E 101° 50.475'	Suburban	Planned Terraces	Have some areas nearby with vegetation, shrubs. Well-managed and clean area.
	Bandar Rinching 4, Hulu Langat	N 02°56.036’ E 101 °50.874'	Suburban	Planned Terraces	Have some nearby areas with vegetation such as rubber estate, shrubs and vegetation. Well-managed and generally clean area.
Kuala Lumpur	Kg. Bharu	N 03010.017’ E 101042.309’	Urban	Scattered Single premises Joined premises	Surrounded with vegetation, trees, shrubs. Not managed properly and unclean.
	Taman Melati	N 03°13.329’ E 101°43.510'	Urban	Planned Terraces	Surrounded with vegetation, trees and shrubs nearby. Properly managed and clean.
	Kelumpuk Cempaka, Keramat	N 03°10.919’ E 101°44.921'	Urban	Planned Flats	Have hill areas nearby with trees and shrubs. Some vegetation. Properly managed and clean area.
	Sentul Utama Flat	N 03°10.088’ E 101°41.882'	Urban	Planned Flats	Have some vegetation’s and shrubssurround/nearby the area. Properly managed and clean area.
	Sri Sabah Flat	N 03°07.005’ E 101°43.515'	Urban	Planned Flats	Have some vegetation and shrubs nearby the area. Properly managed and clean area.
Penang Island	Tingkat Sg. Gelugur	N 05°21.983’ E 100°18.728'	Suburban	Planned Single houses	Surrounded with vegetation, trees and shrubs. Properly managed and clean area.
	Pintasan Bahagia	N 05°19.714’ E 100°17.732'	Suburban	Planned Terraces Single houses	Surrounded with vegetation, trees and shrubs. Properly managed and clean area.
	Permatang Damar Laut	N 05°16.541’ E 100°16.067'	Suburban	Planned Terraces Single houses	Surrounded with vegetation, trees and shrubs. Coastal area. Properly managed and clean area.

iii) Mean number of larvae (per species) per total number of recovered ovitrap for both or either indoor or outdoor:

Mean Number of Larvae/ovitrap =

$$\frac{\text{Total number of larvae}}{\text{Total number of ovitrap collected}} \text{ X } 100$$

iv) Ratio of mosquito species indoor and outdoor

Larval survey

In a locality, a total of 100 premises were randomly surveyed for the presence of *Aedes* sp. Larval surveys were conducted in the same area simultaneously with ovitrapping. The survey was conducted between 8 am to 12 pm. Four assistants were employed to conduct the survey. Repellent containing 2% DEET was applied to the assistants before the survey was conducted as a precautionary measure prescribed under the DOSH (Department of Occupational Safety & Health) requirement for the *Aedes* survey. All potential indoor and outdoor containers or receptacles holding water were inspected. During the survey, water in the containers was poured into white plastic trays. Using disposable pipettes, any mosquito larvae/ pupae present were collected and kept in labelled specimen bottles and recorded accordingly in a prescribed form. If the receptacles were too heavy, big or the water could not be poured out, the water in the receptacles was first agitated using a spoon and the water was removed using a modified pipette. Torchlight was used to examine the receptacles if the condition was very dark such as that in tyres. All the larvae and pupae collected were brought back to the laboratory and identified.

Larval survey data analysis

i) The abundance of *Aedes* mosquito collected by species

ii) The entomological indices:

House (Premises) Index (HI/PI),

= Percentage of the house positive (i.e. Positive for larvae)

$$\text{HI/PI} = \frac{\text{Positive Houses/Premises}}{\text{House/Premises Inspected}} \text{ x}100$$

Container index (CI) =

$$\frac{\text{Containers positive}}{\text{Containers inspected}} \text{ x } 100$$

Breteau Index (BI) =

$$\frac{\text{Total containers positive}}{\text{House inspected}} \text{ x } 100$$

iii) Types of breeding containers and the *Aedes sp.* occurrence

iv) The mean number and ratio of the species indoor and outdoor

RESULTS

Ovitrap- *Aedes* mosquito abundance

In Selangor, a total of 3,046 *Aedes* larvae were collected using ovitrap, comprising of 2,275 *Ae. albopictus* and 771 *Ae. aegypti*. In every locality, more *Ae. albopictus* than *Ae. aegypti* were collected except in Taman Paling Jaya (Figure 1 a). A total of 2,908 larvae comprising 2,033 *Ae. albopictus* and 875 *Ae. aegypti* were collected in Kuala Lumpur. More *Ae. albopictus* than *Ae. aegypti* were collected in every locality except at Sentul Utama Flat, where only *Ae. aegypti* were collected (Figure 1 b). In Penang Island, a total of 646 *Aedes* larvae comprising 574 *Ae. albopictus* and 72 *Ae. aegypti* were collected, with more *Ae. albopictus* in each locality (Figure 1 c). Therefore, based on the total number of *Aedes* species collected during this study, *Ae. albopictus* was more abundant than *Ae. aegypti.*

Ovitrap index and *Ae. albopictus* occurrences

The ovitrap index was more than the transmission threshold of 10% in all localities as summarized in Table 2. This indicates that these localities were at high risk of dengue transmission (Tham, 2000). Table 3 summarizes the mean number of *Ae. albopictus* and *Ae. aegypti* and the ratio of the mosquitoes species for indoor and outdoor according to the study localities.

For Selangor state, in terms of positive ovitrap percentage in Kg. Sg. Ramal Dalam, more single infestation of *Ae. albopictus* was

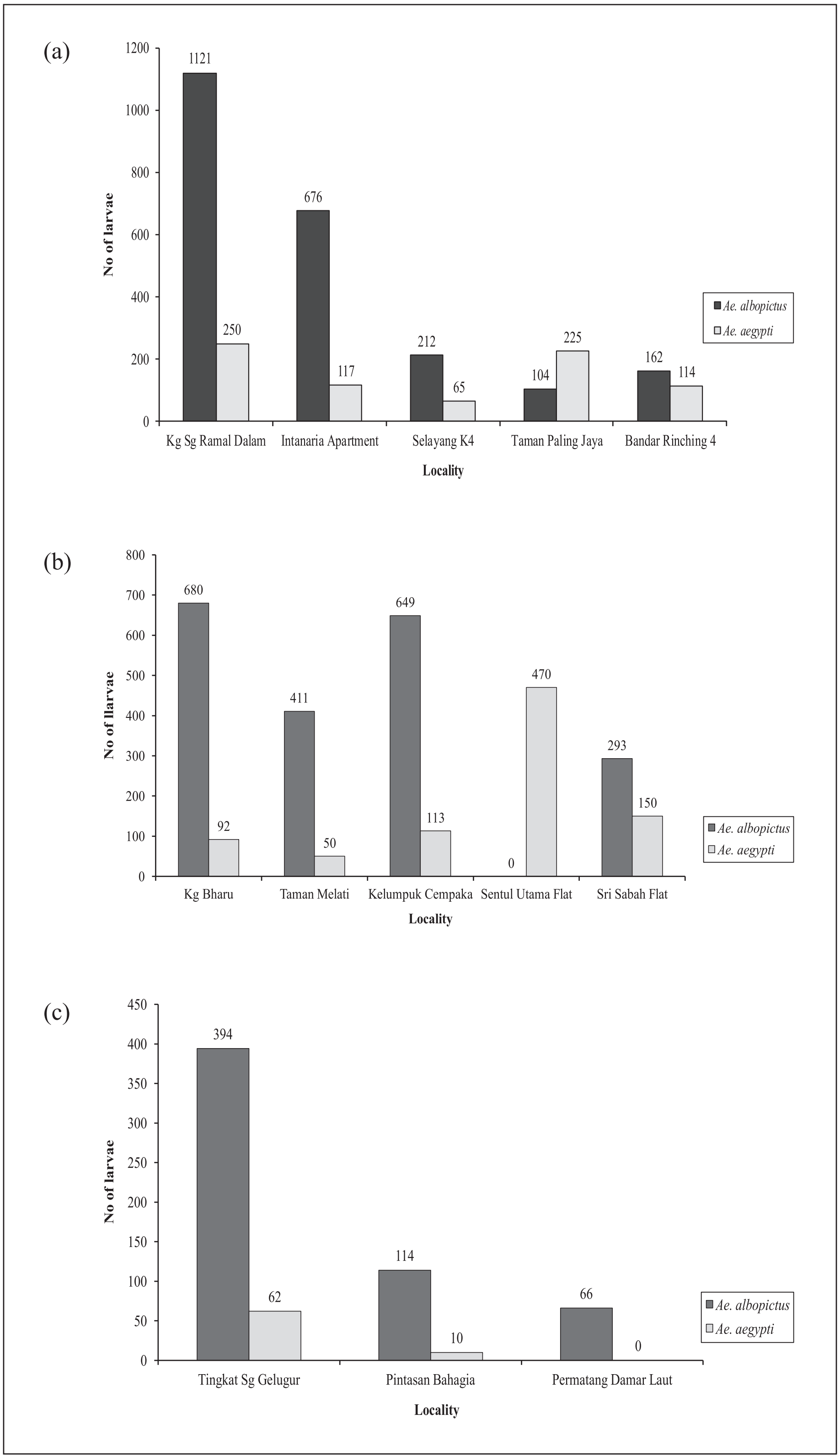

Figure 1. Total number of *Aedes* larvae collected using ovitraps in (a) Selangor (b) Kuala Lumpur (c) Penang Island

Table 2. Ovitrap index (OI) and percentage of indoor and outdoor ovitrap with single and mixed infestation

LOCALITY	OI	INDOOR				OUTDOOR			
		Total	*Ae. albopictus*	*Ae. aegypti*	*Ae. albopictus + Ae. aegypti*	Total	*Ae. albopictus*	*Ae. Aegypti*	*Ae. albopictus + Ae. aegypti*
SELANGOR									
Kg. Sg. Ramal Dalam	70.00	35.71	21.43	2.38	11.90	64.29	38.10	2.38	23.81
Intanaria Apartment	53.33	28.13	3.13	9.38	15. 63	71.88	59.38	3.13	9.38
Selayang K4	51.67	48.39	9.68	25.81	12.90	51.61	38.71	9.68	3.23
Taman Paling Jaya	26.32	40.00	0.00	26.67	13.33	60.00	13.33	46.67	0.00
Bandar Rinching 4	41.07	39.13	0.00	39.13	0.00	60.87	47.83	13.04	0.00
KUALA LUMPUR									
Kg. Bharu	49.15	17.24	3.45	0.00	13.79	82.76	55.17	0.00	27.59
Taman Melati	57.89	30.30	15.15	12.12	3.03	69.70	57.58	0.00	12.12
Kelumpuk Cempaka	62.07	30.56	22.22	2.78	5.56	69.44	50.00	0.00	19.44
Sentul Utama Flat	38.98	52.17	0.00	52.17	0.00	47.83	0.00	47.83	0.00
Sri Sabah Flat	25.42	33.33	0.00	33.33	0.00	66.67	66.67	0.00	0.00
PENANG ISLAND									
Tingkat Sg. Gelugur	40.00	45.83	29.17	8.33	8.33	54.17	37.50	0.00	16.67
Pintasan Bahagia	11.67	28.57	28.57	0.00	0.00	71.43	42.86	0.00	28.57
Permatang Damar Laut	13.33	25.00	25.00	0.00	0.00	75.00	75.00	0.00	0.00

Table 3. Mean number of *Ae. albopictus* and *Ae. aegypti* and the species ratio collected indoor and outdoor using ovitrap

LOCALITY	INDOOR			OUTDOOR			*Ae. albopictus* Indoor : Outdoor
	Ae. albopictus (Mean ± SE)	*Ae. aegypti* (Mean ± SE)	*Ae. albopictus : Ae. aegypti*	*Ae. albopictus* (Mean ± SE)	*Ae. aegypti* (Mean ± SE)	*Ae. albopictus : Ae. aegypti*	
SELANGOR							
Kg. Sg. Ramal Dalam	6.20 ± 2.35	1.43 ± 0.75	4.33 : 1.00	31.16 ± 6.52	6.90 ± 2.60	4.52 : 1.00	1.00 : 5.03
Intanaria Apartment	3.40 ± 1.77	3.13 ± 1.35	1.09 : 1.00	19.13 ± 3.81	0.77 ± 0.46	24.96 : 1.00	1.00 : 5.63
Selayang K4	1.07 ± 0.44	1.40 ± 0.51	1.00 : 1.31	6.00 ± 2.01	0.77 ± 0.41	7.83 : 1.00	1.00 : 5.63
Taman Paling Jaya	1.07± 0.80	6.96 ± 4.04	1.00 : 6.48	2.50 ± 1.74	1.23 ± 0.56	2.03 : 1.00	1.00 : 2.59
Bandar Rinching 4	0	3.00 ± 1.06	0.00 : 81.00	5.59 ± 1.75	1.14 ± 0.97	4.91 : 1.00	0.00 : 162
KUALA LUMPUR							
Kg. Bharu	3.79 ± 2.09	1.97 ± 1.61	1.93 : 1.00	19.00 ± 3.46	1.17 ± 0.51	16.29 : 1.00	1.00 : 5.18
Taman Melati	1.78 ± 0.89	1.00 ± 0.44	1.78 : 1.00	12.10 ± 2.16	0.77 ± 0.49	15.79 : 1.00	1.00 : 7.56
Kelumpuk Cempaka	8.14 ± 3.37	0.93 ± 0.65	8.74 : 1.00	14.24 ± 2.71	2.97 ± 1.57	4.81 : 1.00	1.00 : 1.75
Sentul Utama Flat	0	9.17 ± 3.52	0.00 : 275	0	6.72 ± 2.34	0.00 : 195	0:00
Sri Sabah Flat	0	5.00 ± 2.56	0.00 : 150	10.1 ± 4.10	0:00	293.00 : 0.00	0.00 : 293
PENANG ISLAND							
Tingkat Sg. Gelugur	6.33 ± 4.41	1.20 ± 0.81	5.28 : 1.00	6.8 ± 1.85	0.87 ± 0.64	7.85 : 1.00	1.00 : 1.07
Pintasan Bahagia	0.20 ± 0.15	0	6.00 : 0.00	3.60 ± 2.51	0.33 ± 0.30	10.8 : 1.00	1.00 : 18.00
Permatang Damar Laut	0.77 ± 0.73	0	23.00 : 0.00	1.43 ± 0.89	0	43.00 : 0.00	1.00 : 1.87

found than single infestation of *Ae. aegypti* or mixed infestation. Even though higher percentage of outdoor ovitrap was infested with *Ae. albopictus*, no significant difference was observed between the ovitrap frequency of *Ae. albopictus* infestation outdoor or indoor preferences, [χ^2 (1, N=40) =3.60, p>0.05]. However, based on the indoor to outdoor ratio for the presence of *Ae. albopictus* (1.00: 5.03), more larvae were collected from outdoor ovitrap and the mean number of *Ae. albopictus* was significantly higher in outdoor than indoor (Mann Whitney test, p<0.05). In Intanaria Apartment, single infestation of *Ae. albopictus* outdoor was also higher than infestation of *Ae. aegypti* or mixed infestation. More outdoor ovitraps were infested with *Ae. albopictus* than indoor in this locality [χ^2 (1, N=28) = 9.143 p< 0.05]. These results were proven by a higher ratio of *Ae. albopictus* collected outdoor than indoor (1.00: 5.63) and a significant number of *Ae. albopictus* collected outdoor than indoor (Mann Whitney test, $p<0.05$). In Selayang, the same scenario was found, where higher percentage of ovitrap outdoor with *Ae. albopictus* single infestation was found. The frequency of indoor and outdoor ovitrap infested by *Ae. albopictus* was not significantly different, [χ^2 (1, N=20) = 1.80, $p>0.05$]. However, based on the indoor to outdoor ratio (1.00: 5.63), *Ae. albopictus* was dominant outdoor than indoor and the larvae collected were significantly more outdoor than indoor (Mann Whitney test, $p<0.05$). In Taman Paling Jaya, mixed infestation of *Ae. albopictus* and *Ae.aegypti* was found only in indoor ovitrap (13.33%), and single infestation of *Ae. albopictus* was found only in outdoor ovitrap (13.33%), while single infestation of *Ae. aegypti* was found either indoor or outdoor. Even though there was no significant difference of ovitrap infested frequency for indoor and outdoor by *Ae. Albopictus*, there was also no significant difference of larvae collected indoor and outdoor. However, based on the ratio of indoor to outdoor, *Ae. albopictus* was still found to be dominant outdoor than indoor (1.00: 2.59). In Bandar Rinching 4, although *Ae. aegypti* was found in indoor and outdoor ovitraps, however it was very clear that *Ae. albopictus* infested only outdoor ovitraps, and the ratio of *Ae. albopictus* to *Ae. aegypti* was higher (4.91: 1.00). Therefore, it was clear that *Ae. albopictus* preferred outdoor in this locality. Based on this analysis, it was determined that *Ae .albopictus* preferred to breed outdoor than indoor in all study localities in Selangor state.

In the Federal Territory of Kuala Lumpur, each locality recorded higher percentage of positive outdoor ovitraps than indoor except for Sentul Utama Flat, Kuala Lumpur, where the percentage of positive ovitrap indoor (52.17%) was slightly higher than the outdoor (47.83%). In Kg. Bharu, the outdoor ovitrap was significantly more infested with *Ae. albopictus* than indoor, (χ^2 = 12.448 $p<0.05$) and the ratio of *Ae. albopictus* outdoor was higher than indoor (1.00: 5.18), while the mean number larvae collected outdoor was significantly higher than indoor (Mann Whitney test, $p<0.05$). In Taman Melati, higher percentage of indoor ovitrap was positive with *Ae. albopictus* (15.15%) compared to *Ae. aegypti* (12.12%) or mixed infestation (3.03%). The outdoor ovitraps in Taman Melati also recorded a higher percentage of single infestation of *Ae. albopictus* (57.58%) than mixed infestation, with no ovitrap containing single infestation of *Ae. aegypti*. *Aedes albopictus* was found to prefer outdoor ovitrap significantly based on the frequency of ovitrap infested by the species, (χ^2 =9.97, $p <0.05$), with the ratio of infestation by *Ae. albopictus* (1.0: 7.56, indoor: outdoor) and the mean number of larvae collected was significantly higher in outdoor ovitrap than indoor (Mann Whitney test, $p<0.05$). For Kelumpuk Cempaka in Keramat, both indoor (22.22%) and outdoor (50%) recorded higher percentage of *Ae. albopictus* infestation than *Ae. aegypti* or by mixed infestation of both species. No ovitrap with a single infestation of *Ae. aegypti* in outdoor ovitraps was found in this locality. Moreover, it was also found that *Ae. albopictus* infested more outdoor ovitrap than indoor (χ^2=6.429, $p<0.05$). Higher ratio of larvae was collected outdoor (1.00: 1.75) and significantly more larvae of *Ae. albopictus* were collected outdoor than indoor (Mann Whitney test $p<0.05$). In Sentul Utama Flat, only *Ae. aegypti* was recorded in either indoor or outdoor ovitraps, whereas in Sri Sabah Flat, only *Ae. aegypti* was found in indoor ovitrap and *Ae. albopictus* in outdoor ovitrap with no mixed infestation. Therefore, based on these analyses, it was determined that *Ae. albopictus* preferred to breed outdoor than indoor in all study localities in Kuala Lumpur except in Sentul Utama Flat, in which only *Ae. aegypti* was found.

In Penang Island, all localities recorded higher percentage of *Ae. albopictus* single infestation both indoor and outdoor. No *Ae. aegypti* infestation or mixed infestation of *Ae. albopictus* and *Ae. aegypti* were recorded in Permatang Damar Laut. Although there was no significant difference in the frequency of outdoor and indoor ovitrap infested with *Ae. albopictus*, the mean number and the ratio of total number of *Ae. albopictus* larvae collected indoor and outdoor in all this localities showed a higher ratio of *Ae. albopictus* in outdoor than indoor (1.00: 1.07, 1.00: 18.00 and 1.00: 1.87, respectively). Therefore, it can be concluded that *Ae. albopictus* still prefer to breed outdoor rather than indoor at these localities.

Larval survey – *Aedes* mosquito abundance

A total of 706 *Aedes* larvae comprising 504 *Ae. albopictus* and 202 *Ae. aegypti* larvae were collected in localities in Selangor. *Aedes albopictus* was more abundant than *Ae. aegypti* in Kg. Sg. Ramal Dalam and Selayang K4. In Intanaria Apartment, no larvae were collected, whereas only *Ae. albopictus* was collected in Taman Paling Jaya. In Bandar Rinching 4, more *Ae. aegypti* than *Ae. albopictus* was collected (Figure 2a). In Kuala Lumpur, a total of 1,288 *Aedes* larvae were collected comprising 838 *Ae. albopictus* and 450 *Ae. aegypti.* Only Kg. Bharu and Taman Melati were positive with the mosquitoes, both recorded more *Ae. albopictus* than *Ae. aegypti* (Figure 2b). In Penang Island, a total of 1,307 larvae were collected comprising 460 *Ae. albopictus* and 847 *Ae. aegypti.* More *Ae. albopictus* was collected than *Ae. aegypti* only in Pintasan Bahagia (Figure 2c).

Entomological indices, type of breeding containers and *Aedes albopictus* occurrences

Table 4 summarizes the House Index (HI), Container Index (CI) and Breteau Index (BI) for each locality. For Selangor, except Kg. Sg. Ramal, showed a low to moderate HI (1-10%), whereas in Bangi, all indices could not be determined since no positive houses were found. The BI was also moderate (≥5%, ≤50%) for the three localities except for Taman Paling Jaya, which was at a very low risk of dengue transmission (≤5%). In Kuala Lumpur, only two localities could be determined for all indices; Kg. Bharu and Taman Melati. Both HI and CI showed a high risk of dengue transmission (≥10%, ≥5% respectively), whereas the BI for both localities was at moderate risk for dengue transmission (≥5% ≤50%). All three localities in Penang Island showed a moderate HI (≤10%), high rate of CI (≥5%), and low to moderate rate for risk of dengue transmission based on BI (≤5%, ≤50%).

Aedes albopictus has a broad range of breeding habitats ranging from natural sites such as bamboo stumps, tress holes, leaf axils, bromeliads plants to various artificial containers that can collect water (Hawley, 1988; Paupy *et al.*, 2009). Throughout this study, although both indoor and outdoor premises were investigated for the presence of the mosquitoes, more outdoor containers were available and inspected. A range of natural and artificial containers were inspected and found to be positive with the *Ae. albopictus* and/or *Ae. aegypti* immature. Each locality contained similar types of breeding sites such as plastic containers, tyres, water storage container, and others. Similar to the ovitraps, each positive container was infested either by single infestation of *Ae. albopictus* or *Ae. aegypti* or mixed infestation of both species.

Tables 5 (a) summarize the type of containers inspected and *Aedes* larval occurrence in both indoor and outdoor in Selangor. In Kg. Sg. Ramal Dalam, a total of 51 containers were inspected and 18 containers were found positive. The most common containers with water inspected were plastic containers (35.29%), followed by paint tin (17.64%), where both containers also recorded the highest positive containers (22% respectively). Only single infestation of *Ae. albopictus* and mixed infestation of *Ae. albopictus* and *Ae. aegypti* were recorded in this locality. *Aedes albopictus* infested more outdoor containers than indoor (χ^2 =14.22, p<0.05), and the ratio of larvae collected was higher for outdoor (1.00: 94.67), with the number of *Ae. albopictus* larvae significantly higher for outdoor than indoor (Mann Whitney, p<0.05). In Intanaria Apartment, a total of 20 containers were inspected and the plastic containers remained as the main breeding site, followed by flower pots and tyres. However, none of them was found to be positive with mosquito. In Selayang, six types of breeding sites were inspected, which comprised of 13 inspected containers and 11 positive containers. The main container available and the highest positive container

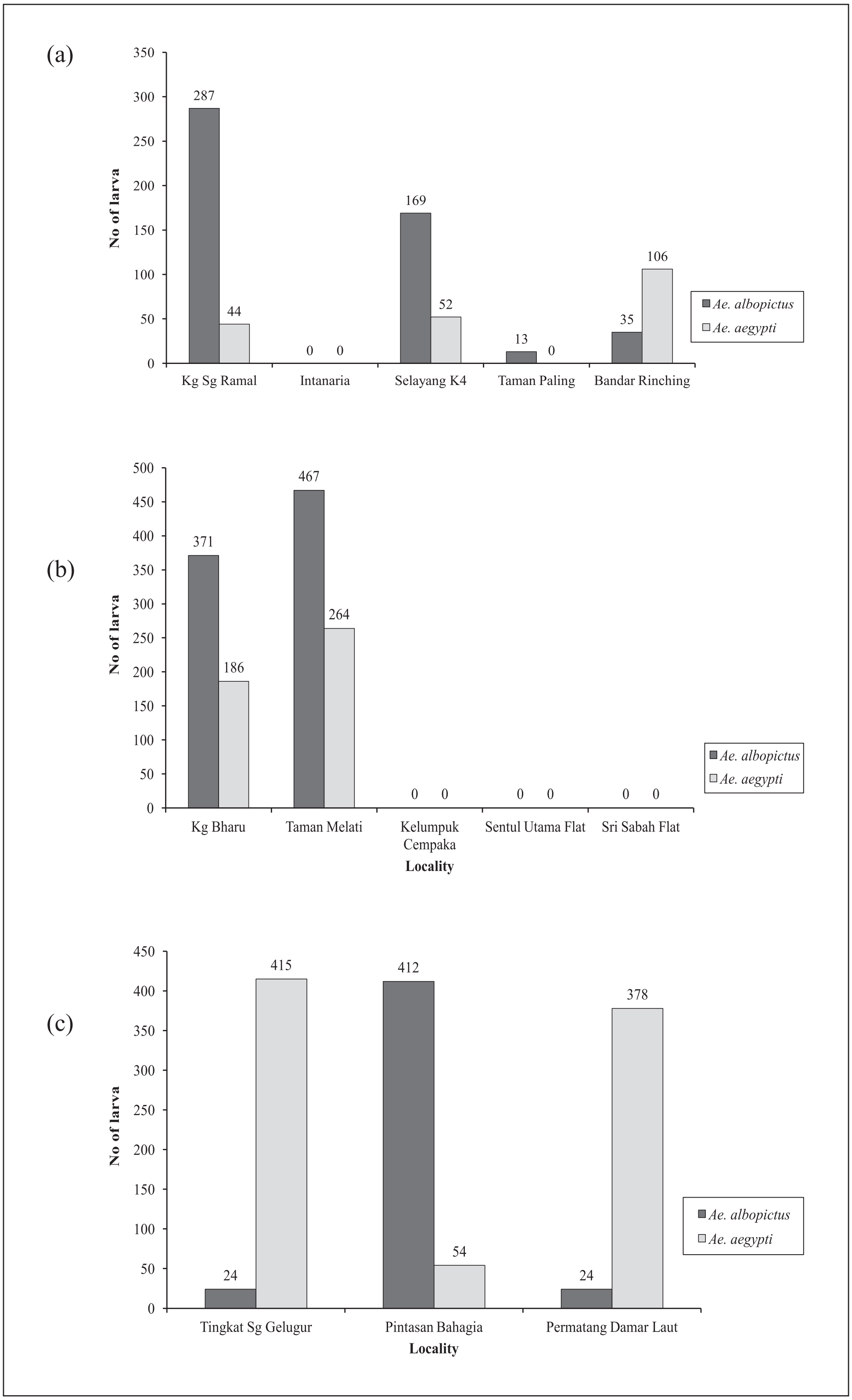

Figure 2. Total number of *Aedes* larvae collected using larval survey method in (a) Selangor (b) Kuala Lumpur (c) Penang Island

Table 4. The House index (HI), Container Index (CI) and Breteau Index (BI) for each study localities

Locality	No. house positive	No. container inspected	No. container positive	HI %	CI %	BI
SELANGOR						
Kg. Sg. Ramal Dalam	17	51	18	17	35.29	18
Intanaria Apartment	0	20	0	0	0.00	0
Selayang K4	7	13	11	7	84.62	11
Taman Paling Jaya	1	9	1	1	11.11	1
Bandar Rinching 4	8	18	6	8	33.33	6
KUALA LUMPUR						
Kg. Bharu	18	85	27	18	31.76	27
Taman Melati	10	24	15	10	62.50	15
Kelumpuk Cempaka	0	30	0	0	0.00	0
Sentul Utama Flat	0	1	0	0	0.00	0
Sri Sabah Flat	0	3	0	0	0.00	0
PENANG ISLAND						
Tingkat Sg. Gelugur	4	18	3	4	16.67	3
Pintasan Bahagia	4	86	6	4	6.98	6
Permatang Damar Laut	6	94	6	6	6.38	6

Table 5. Percentage occurrence of *Ae albopictus* by container categories, indoors and outdoors

(a) Selangor

Location	Container category	% occurrence of *Ae. albopictus* by container categories			
		Kg Sg Ramal Dalam	Selayang	Taman Paling Jaya	Bandar Rinching 4
Indoor	garbage bin				16.67
	helmet				16.67
	mat		9.09		
	plastic container				16.67
	tyre	5.56			
Outdoor					
	bottle	11.11		100.00	
	cup	5.56			
	flower pot base		18.18		
	flower watering can				16.67
	garbage bin cover	5.56			
	glass cooker				16.67
	mat		9.09		
	paint tin	22.22			
	plastic container	22.22	27.27		
	table		9.09		
	tyre	11.11			
	water storage container	11.11	18.18		

(b) Kuala Lumpur

	Container category	% occurrence of *Ae. albopictus* by container categories	
		Kg Bharu	Taman Melati
Indoor	aquarium	7.41	
	plastic container	11.11	6.67
	water storage containers	3.70	
Outdoor	canvas	3.70	
	coconut	6.67	
	drawer	3.70	6.67
	flower pot	3.70	
	flower pot base	6.67	
	flower sheath of palm tress	6.67	
	garbage bin	13.33	
	mat	7.41	
	motorcycle battery compartment	3.70	
	paint tin	14.81	6.67
	paper cup	3.70	
	plastic container	7.41	20.00
	plastic divider	3.70	
	plastic liner	3.70	
	toilet bowl	3.70	
	toilet flush	3.70	
	tyre	7.41	6.67
	water storage container	7.41	

(c) Penang Island

	Container category	% occurrence of *Ae. albopictus* by container categories		
		Tingkat Sg Gelugur	Pintasan Bahagia	Permatang Damar Laut
Indoor	plastic container	33.33		33.33
	plastic pail			16.67
	tyre			16.67
	water storage container	33.33		33.33
Outdoor	garbage bin		16.67	
	flower pot		16.67	
	flower watering can		16.67	
	paint tin		16.67	

inspected was still plastic containers (30.76% and 27.27%, respectively). *Aedes albopictus* infested more outdoor containers than indoor (χ^2 = 4.50, p<0.05], and the ratio of outdoor was higher than indoor (1.00: 6.35; indoor: outdoor). In Taman Paling Jaya, only 9 containers were available outdoor and only one bottle was positive with a single infestation of *Ae. albopictus*. In Bandar Rinching 4, 18 containers were inspected with identified as 6 positive with equal positivity (16.67%), and plastic containers were still the most abundant breeding site (22.22%). Even though there was no significant difference between the frequency of indoor and outdoor containers infested by *Ae. albopictus* (χ^2(= 0.200, p>0.05) and the mean number of the 2 species, the ratio was still higher outdoor than indoor (indoor: outdoor=1.00: 4.00). Therefore, the larval survey conducted in Selangor showed that *Ae. albopictus* preferred to breed outdoor than indoor.

Kampung Bharu recorded a very wide range of potential containers (85 containers) and 27 positive breeding containers throughout the study. Plastic containers remained the highest containers with water inspected (27%) and the highest positive containers (18.52%, 11.11% indoor and 7.41% outdoor). *Aedes albopictus* preferred to breed outdoor than indoor (χ^2 =15.696, p<0.05), with the ratio of 1.00: 19.61 (indoor: outdoor). In Taman Melati, a total of 24 potential containers were inspected, with 15 of them were positive. The main containers available and positive with breeding were still plastic containers (37.5% and 26.67%, respectively). In this locality, the species was also found to prefer breeding outdoor than indoor with the frequency of outdoor containers significantly more than in indoor containers (χ^2(=8.33, p<0.05), and there was a significant difference in the number of *Ae. albopictus* larvae collected outdoor compared to indoor (Mann Whitney test, p<0.005) with a ratio of 1.00: 232.50 (indoor: outdoor). In the residential flat areas, only a few potential containers could be found and none of them was positive with mosquitoes. 30 outdoor containers were inspected in Kelumpuk Cempaka, Keramat, 1 in Flat Sentul Utama and 3 in Flat Sri Sabah which comprised the flower pot bases, paint tins, plastic bags, plastic containers, tyres, and water storage containers. Plastic containers remained the most potential containers inspected (70%). Based on these results, it was also determined that *Ae. albopictus* preferred to breed outdoor in the localities under study in Kuala Lumpur. The types of containers and *Aedes* larval occurrence in both indoor and outdoor in Kuala Lumpur is summarized as in Table 5 (b), whereas the mean number and the ratio of the species is summarized in Table 6.

The type of containers and *Aedes* larval occurrence in both indoor and outdoor in Penang Island are summarized in Table 5c (whereas the mean number of the mosquito species and the ratio of both indoor and outdoor are summarized in Table 6. In Tingkat Sungai Gelugur, *Ae. albopictus* was only found indoor mixed with *Ae. aegypti* with the ratio of *Ae. albopictus*:*Ae. aegypti* = 1.00: 17.29, indicating that *Ae. aegypti* was more abundant indoor than outdoor. From the 18 containers inspected, plastic containers were found to be the most abundant containers inspected (38%). with the same positivity percentage (33.33%) with the water storage containers. A wide range of containers were inspected in Pintasan Bahagia (86 containers inspected with 6 positive). Other than plastic containers, garbage bins were found to be the most abundant breeding containers (19.77%). Only outdoor containers were positive (with equally positivity percentage 16.67%) with

Table 6. Mean number of *Ae. albopictus* and *Ae. aegypti* and the species ratio collected indoor and outdoor using larval survey method

LOCALITY	INDOOR			OUTDOOR			*Ae. albopictus* Indoor : Outdoor
	Ae. albopictus (Mean ± SE)	*Ae. aegypti* (Mean ± SE)	*Ae. albopictus : Ae. aegypti*	*Ae. albopictus* (Mean ± SE)	*Ae. aegypti* (Mean ± SE)	*Ae. albopictus : Ae. aegypti*	
SELANGOR							
Kg. Sg. Ramal Dalam	3.00 ± 0.0	13.00 ± 0.00	1.00 : 4.33	15.78 ± 2.71	3.87 ± 0.79	9.16 : 1.00	1.00 : 94.67
Intanaria Apartment	0	0	0	0	0	0	0
Selayang K4	23.00 ± 0.00	22.00 ± 6.00	1.00 : 1.91	16.22 ± 2.62	2.67 ± 0.88	18.25 : 1.00	1.00 : 6.35
Taman Paling Jaya	0	0	0	1.87 ± 1.87	0	13.00 : 0.00	0.00 : 13.00
Bandar Rinching 4	2.23 ± 0.88	21.00 ± 16.38	1.00 : 12.00	14.00 ± 11.00	11.00 ± 8.00	1.27 : 1.00	1.00 : 4.00
KUALA LUMPUR							
Kg. Bharu	6.00 ± 3.05	23.67 ± 19.17	1.00 : 3.94	18.57 ± 4.33	10.45 ± 3.58	3.07 : 1.00	1.00 :19.61
Taman Melati	2.00 ± 0.00	19.10 ± 7.73	1.00 : 86.00	46.50 ± 13.50	23 ± 21.33	5.05 : 1.00	1.00 : 232.5
Kelumpuk Cempaka	0	0	0	0	0	0	0
Sentul Utama Flat	0	0	0	0	0	0	0
Sri Sabah Flat	0	0	0	0	0	0	0
PULAU PINANG							
Tingkat Sg. Gelugur	12.00 ± 11.00	138.98 ± 122.98	1.00 : 17.29	0	0	0	24.00 : 0.00
Pintasan Bahagia	0	0	0	103 ± 72.88	18.00 ± 10.11	7.63 : 1.00	0.00 : 412
Permatang Damar Laut	24.00 ± 0.00	63.00 ± 29.4	1.00 : 15.75	0	0	0	24.00 : 0.00

Ae. albopictus and *Ae. aegypti* with a significantly higher number and ratio of *Ae. albopictus* (7.63: 1.00 = *Ae. albopictus*: *Ae. aegypti*). In Permatang Damar Laut, 94 containers were inspected with only 6 positive. At this locality, only indoor containers were positive with *Ae. albopictus* and *Ae. aegypti* with the number of *Ae. aegypti* larvae significantly higher than *Ae. albopictus* and the ratio of *Ae. albopictus* to *Ae.aegypti* was 1.00 : 15.75. The plastic containers (45.75%) remained the major containers inspected. Plastic containers and water storage containers (33.33% respectively) were recorded most positivity percentage with the mosquito species. Through this larval survey, it was determined that in localities studied in Penang Island, *Ae. albopictus* still preferred breeding outdoor than indoor based on higher ratio of outdoor breeding compared to *Ae. aegypti*.

DISCUSSIONS

This study showed that ovitrap remains as a sensitive, economical and reliable tool to detect/survey the abundance of container breeder such as *Ae. aegypti* and *Ae. albopictus* since we have collected more mosquitoes using ovitrap than the conventional larval survey. Similar findings were reported by Lee (1992), where the author found that ovitrapping was a more useful and sensitive technique to monitor or survey *Aedes* when larval survey showed low infestation rate of *Aedes*. Dengue and yellow fever vectors surveillance in Brazil also found that ovitrap was more effective than the larval survey (Marques *et al.*, 1993; Braga *et al.*, 2000; de Melo, *et al.*, 2012).

The effectiveness of larval survey is dependent on the experience and technical expertise of the collectors. It is more demanding as it is time consuming and is constrained by several factors such as the hot weather or rain which may stop/ demotivate the collectors to conduct the search. In addition, larval survey is more labour intensive, e.g. in this study, 4 trained collectors were required to perform the survey. Larval survey is also dependent on the individual effort of the collectors to search all potential breeding containers. A collector may miss the containers, especially if they are indoor since house owners at times are not cooperative. The ovitraps are easier to handle, can be placed at the study site for not more than 7 days and needed only one to two persons to place/collect the ovitraps in the field. The placement of ovitraps is not a problem since outdoor and indoor have been defined clearly. In this study, both survey methods were performed indoor (inside the house or have 4 walls) and outdoor (outside of the premise). In the apartment and residential flat areas, very few breeding containers were found. The management may have properly discarded the outdoor containers due to dengue outbreak in that area. Most of the house owners also did not permit larval surveys to be conducted inside their houses. The corridors seemed to be very clean and well maintained.

On the other hand, through the larval survey, we were able to obtain more information on the breeding foci preference of the species, either in natural or artificial containers. Such information can be used by vector control personnel to educate the public in order to obtain their full cooperation. During the survey, the weather was quite dry and hot with a very low rainfall. Therefore, not much information could be gathered on the natural breeding foci even though we had inspected some banana leaf axils and bromeliads plant in the localities. Most localities had various breeding containers and the plastic containers were usually the predominant breeding site. Similar finding was reported by Chareonviriyaphap *et al.* (2003), who found that plastic containers was one of the main *Ae. albopictus* larval breeding containers in Thailand during the dry period. Recently, during the surveillance of chikungunya vectors in Kelantan state, we found plastic containers to be most abundant (Rozilawati *et al.*, 2011). This information will be useful for vector control personnel to promote public awareness for the purpose of source reduction. The occurrences of *Ae. albopictus* and *Ae. aegypti* as mixed infestation of both species either in ovitraps or in other breeding containers indoor or outdoor in this study indirectly indicated that both species can oviposit in the same containers, as reported by many other researchers (Chan *et al.*, 1971; Yap & Thiruvengadam, 1979; Lee, 1992; Chang & Jute, 1994; Chen, *et al.*, 2006a,b; Norzahira *et al.*, 2011). In this study, it was also determined that the entomological indices (house index, container index and Breteau index) indicated mostly low to moderate risk of dengue transmission. However, the ovitrap index had indicated that the localities were at high risk of dengue transmission (OI> 10%) (Tham, 2000). Therefore, preventive measures should be instituted by the vector control program.

All localities in this study indicated that *Ae. albopictus* was found predominantly outdoors (based on the frequency of *Ae. albopictus* occurrences, ratio, and mean number of larvae) in the all localities except in Sentul Utama Flat, where only *Ae. aegypti* larvae were collected through the ovitrapping and larval survey. This finding is similar to the studies conducted in Selangor, Kuala Lumpur, and Penang Island using ovitrap and larval survey, which indicated that *Ae. albopictus* was more abundant outdoor than indoor (Yap, 1975; Chen *et al.*, 2005; Chen *et al.*, 2006 a,b; Rozilawati *et al.*, 2007; Wan-Norafikah *et al.*, 2009; Saleeza *et al.*, 2011). Although the abundance of *Ae. albopictus* in Penang Island was different in ovitrap and larval survey, i.e. ovitrap collected more *Ae. albopictus*, whereas more *Ae. aegypti* was collected in larval survey, based on the ratio of both species, *Ae. aegypti* was still predominantly indoor compared to *Ae. albopictus* (in Tingkat Sg Gelugur and Permatang Damar Laut), while *Ae. albopictus* was more abundant outdoor than *Ae. aegypti* (in Pintasan Bahagia). The ovitrap showed higher ratio of *Ae. albopictus* outdoor than indoor in all the localities in this state. It was also reported by Lee (1991, 1992) during ovitrap and larval survey in urban and suburban areas in peninsular Malaysia, that even though that both *Ae. aegypti* and *Ae. albopictus* can be found indoor and outdoor, however it was indicated that *Ae. aegypti* was more dominant indoor and *Ae. albopictus* outdoor. *Aedes albopictus* was also found predominant in suburban than urban areas. In this study, there was no evidence that *Ae albopictus* was replaced by *Ae aegypti*, in contrast to a study conducted in suburban areas in Selangor (Lee, 1992).

Therefore, it can be concluded that there is currently no change of *Ae. albopictus* breeding preference even though the species can also be found indoors in all study localities.

The current vector control programme should be integrated to control *Ae. albopictus*, the main chikungunya vector, together with the main dengue vector, the indoor breeding *Ae. aegypti*. The breeding preference behaviour of *Ae. albopictus* is one of the important aspects to be investigated, as any changes of the breeding behaviour may ultimately affect control measures. It is strongly suggested that the surveillance of the mosquitoes to be continuously performed using standard ovitrap and larval survey.

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