Larval Distributions and breeding habitats of Aedes aegypti and Ae. albopictus in Kuala Terengganu

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Larval Distributions and breeding habitats of *Aedes aegypti* and *Ae. albopictus* in Kuala Terengganu

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Abstract. *Aedes aegypti* and *Ae. albopictus* play an important role in spreading dengue and chikungunya virus in Malaysia. Currently dengue fever is still the most important threat to this country. The number of dengue cases are on the rise in some states despite the efforts made by the government to keep the surroundings of houses clean and free from *Aedes* breeding sites. This study was carried out to determine the breeding sites of *Aedes* in Kuala Terengganu. Samplings were carried out between August 2009 until February 2010 at six study sites from both the urban and rural areas. Samples collected were counted and the distribution classes (class 1 (C1) to class 5 (C5)) were determined. Water tank, glass container and used paint bucket were found to be the most preferable breeding sites in the study areas. Many *Ae. aegypti* larvae were collected in the urban areas and the highest number of *Ae. albopictus* larvae were collected from the rural areas. As for the larval distributions, *Ae. aegypti* recorded a frequent distribution (C4: 60.1-80%) in urban areas while *Ae. albopictus* recorded infrequent distribution (C2: 20.1-40%). Frequent distributions of *Aedes* larvae were recorded when relative humidity is high. Apart from environmental factors, human density also could affect the distributions of *Aedes* larvae in the urban area.

INTRODUCTION

Since the 19th century, mosquito-borne disease has been endemic in 112 countries and causes negative effect to human health. Mosquitoes are vectors to many types of diseases and millions of people died because of the diseases brought by mosquitoes (Becker *et al*., 2003). There are approximately 3500 species of mosquitoes worldwide and some have become important vectors especially in subtropical region (Mandal *et al*., 2008). Villages, agricultural farms and urban areas are believed to provide most breeding sites for mosquitoes (Vythilingham *et al*., 2005). In Malaysia, there are about 415 mosquito species recorded with 75 species that are endemic (Foley *et al*., 2007). A few of them such as *Aedes* spp., *Anopheles* spp. and *Culex* spp are vectors to many mosquito-borne diseases. Among those, *Ae. aegypti* and *Ae. albopictus* are two most important species that transmit dengue virus. In Malaysia, the number of dengue fever and death keep arising every year. In 2010 there were 46171 dengue cases with 134 death reported by the Ministry of Health (KKM), Malaysia. Most number of dengue cases were recorded in Selangor (16367 cases) followed by Johor (4406 cases), Sarawak (4240 cases) and Wilayah Persekutuan Kuala Lumpur and Putrajaya (4205 cases). Terengganu recorded 1472 cases which was 52% higher than the previous year, 2009 (968 cases). In the latest report for the year 2015, as of 11th July 2015, 62,648 of dengue cases had been reported with 173 deaths. This is 148% higher compared to the same reporting period of 2010 (25301 cases) (KKM, 2015).

It is crucial to control the number of breeding habitats of *Aedes* mosquitoes in order to reduce the number of dengue cases. *Aedes* can breed in many artificial and natural containers (Chen *et al.*, 2009; Wan-Norafikah *et al.*, 2012; Saifur *et al.*, 2013; Saleeza, 2013). To date, various studies on *Aedes* breeding habitats had been conducted in Malaysia (Chen *et al.*, 2009; Wan-Norafikah, 2012; Dieng *et al.*, 2012; Saifur *et al.*, 2013; Saleeza *et al.*, 2013; Nazri *et al.*, 2013; Muhammad-Aidil *et al.*, 2015). However, only few studies have been conducted in the East Coast of Peninsular Malaysia especially in the state of Terengganu. Hence, we conducted this research to determine the distribution of *Ae. aegypti* and *Ae. albopictus* focusing in the areas of Kuala Terengganu. It is hoped that the information from this research will provide better understanding about the distribution of mosquitoes in Kuala Terengganu and eventually could help in organizing a better *Aedes* control programme in the future.

MATERIALS AND METHODS

Study sites

This study was conducted in selected rural and urban areas in Kuala Terengganu. Each area was divided into three study sites. In rural area, Kampung Durian Burong (KDB; 5°18.1852 N, 103°6.52E), Kampung Kubang Jela (KKJ; 5°19.4172N, 103°5.0052E) and Kampung Chabang Tiga (KCT; 5°18.9772N, 103°7.3232E) were chosen while three study sites in urban areas were Kampung Cina (KC; 5°20.0052N, 103°7.9682E), Batu Burok (BB; 5°19.6072N, 103°9.1182E) and Taman Syahbandar (TSB; 5°20.2372N, 103°8.3602E). The sampling activities at each sampling site were conducted on certain areas outside buildings and premises such as at the construction sites, dump sites, drainage areas and bushes.

Sampling methods

All containers found outside of housings and buildings in the sampling areas were inspected to determine the presence of mosquitoes' larvae. Dipper and pipette were used to collect samples of water containing larvae from the breeding containers. Samples were then transferred into bottles and brought back to laboratory. In the laboratory, larvae were identified (if possible) using keys provided by Rueda (2004). Unidentified larvae were reared in the laboratory under normal room temperature (27±3°C) and humidity (75±5)% and were given fish food (Double Red, Sanyu TOKO Aquaria Fish Food) until reaching 3rd larval instars stage or until they emerged as adult mosquitoes before the identification process was performed. This study was conducted for seven months beginning from August 2009 until February 2010.

Data analysis

Larval distributions in the study area were analyzed using formula provided by Dzieczkowski (1972):

Distribution:
C = (n/N) x 100%
[C = distribution; n = total number of breeding sites of each species; N = total number of all breeding sites]

Distribution class:
C1: 'Sporadic' (0-20%)
C2: 'Infrequent' (20.1-40%)
C3: 'Moderate' (40.1-60%)
C4: 'Frequent' (60.1-80%)
C5: 'Constant' (80.1-100%)

Statistical analysis

The difference in the total number of individual for each species of mosquito at each study area were analyzed using a Chi-square test. The relationship between the distributions class of *Ae. aegypti* and *Ae. albopictus* with the physical parameters were analyzed using Self-Organizing Map (SOM) Toolbox 2 (Clustering and Analysis, MATLAB 7.0).

RESULTS

Table 1 shows the number of breeding sites of *Aedes* spp. found in urban and rural areas. TSB and BB recorded positive breeding sites for *Aedes* sp., while there was no breeding site found at KC. At both TSB and BB, *Aedes* larvae were found breeding in glass container, plastic bottles and in water tank. *Aedes aegypti* recorded the highest number in all breeding habitats. In contrast, only KDB which represents the rural area recorded positive for mosquitoes' breeding habitat. The mosquitoes were found breeding in used safety helmets, paint bucket and coconut shells. The distribution percentage of *Ae. aegypti* (71.25%) was higher than the distribution percentage of *Ae. albopictus* (28.75%) at TSB (Figure 1). Meanwhile at BB, the distribution of *Ae. aegypti* (59.09%) recorded a higher percentage compared to the distribution percentage of *Ae. albopictus* (40.91%). At KDB, *Ae. albopictus* (66.67%) recorded a higher distribution percentage than *Ae. aegypti* (33.33%).

Distribution class of *Ae. aegypti* in urban areas fall under class C4 (frequent: 60.1-80%) while the distribution class of *Ae. albopictus* was C2 (infrequent; 20.1-40%) (Table 2). In rural areas, the distribution class of *Ae. aegypti* and *Ae. albopictus* fall under C2 (infrequent; 20.1-40%) and C4 (frequent: 40.1-

Table 1. List of breeding sites found at urban and rural areas

Sampling areas	Locations	Breeding sites	Species	No. of individuals
Urban	KC	*Nil*	–	–
	TSB	Glass containers	*Ae. aegypti*	32
			Ae. albopictus	8
		Plastic bottles	*Ae. aegypti*	25
			Ae. albopictus	5
		Coconut shells	*Ae. aegypti*	0
			Ae. albopictus	10
	BB	Glass containers	*Ae. aegypti*	17
			Ae. albopictus	12
		Water tanks	*Ae. aegypti*	35
			Ae. albopictus	24
			Total	**168**
Rural	KDB	Safety helmet	*Ae. aegypti*	0
			Ae. albopictus	18
		Paint buckets	*Ae. aegypti*	16
			Ae. albopictus	25
		Coconut shells	*Ae. aegypti*	18
			Ae. albopictus	21
		Plastic containers	*Ae. aegypti*	0
			Ae. albopictus	8
	KKJ	*Nil*	–	–
	KCT	*Nil*	–	–
			Total	**106**

*KC: Kampung Cina; TSB: Taman Syahbandar; BB: Batu Burok; KDB: Kampung Durian Burung: KKJ: Kampung Kubang Jela; KCT: Kampung Chabang Tiga.

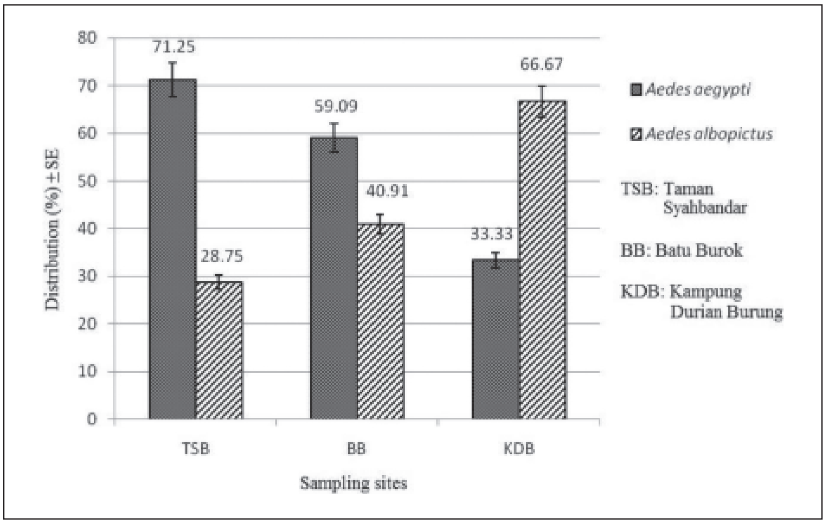

Figure 1. Percentage of larval distribution of *Ae. aegypti* and *Ae. albopictus* at three study sites (data was calculated using formula provided by Dzieczkowski (1972)). Both TSB and KDB which represent the urban and rural areas recorded frequent (class C4) of larval distribution respectively.

Table 2. Distribution class of *Aedes* spp. in urban and rural areas

Area	Species	Distribution (%)	Distribution class
Urban	*Ae. aegypti*	64.48	C4
	Ae. albopictus	35.12	C2
Rural	*Ae. aegypti*	33.33	C2
	Ae. albopictus	66.67	C4

60%), respectively (Table 2). There was a significant difference between the total numbers of *Ae. aegypti* and *Ae. albopictus* larvae in urban and rural areas ($\chi^2_{(1, N=274)}$ = 27.93, p <0.01). Only two distribution classes were identified during current study which were C2 (sporadic distribution) and C4 (frequent distribution) (Table 3; Figure 2). Class C2 was recorded when the temperature was high while humidity and rainfall were low in the study area. In contrast, class C4 was recorded when the humidity was high while both temperature and rainfall were low.

DISCUSSION

In the rural area, *Ae. albopictus* was found to be more abundant compared to *Ae. aegypti*. This is because rural areas can produce many types of breeding habitats for *Ae. albopictus* compared to the urban areas (Saifur *et al.*, 2013) and the female *Ae. albopictus* can discriminate breeding habitats for egg depositions in order to improve survival of their offspring (Zhang & Lei, 2008; Obenauer *et al.*, 2010). Frequent distribution of *Ae. aegypti* was recorded in urban areas. Two urban areas, Taman Syahbandar and Batu Burok recorded high population density and this indirectly resulted in the increasing population of *Aedes* sp. in the urban areas. Both places are experiencing rapid development in terms of facilities and infrastructures. A study by Kwa (2008) suggested that rapid development will contribute to increase in *Aedes* sp. population due to creation of new breeding sites.

Table 3. Physical parameters at each site during data collection

No. of sampling	Location	Temperature (°C)	Humidity (%)	Rainfall (mm)
1	KC	27.8	73.0	0.0
2	TSB	28.2	80.3	0.2
3	TSB	28.4	79.9	0.0
4	BB	26.6	87.2	0.2
5	BB	27.0	83.7	73.8
6	KDB	26.4	83.2	0.0
7	KKJ	27.3	78.5	0.0
8	TSB	27.7	77.7	0.0
9	KBD	27.4	77.2	0.0
10	KCT	27.6	77.2	0.0

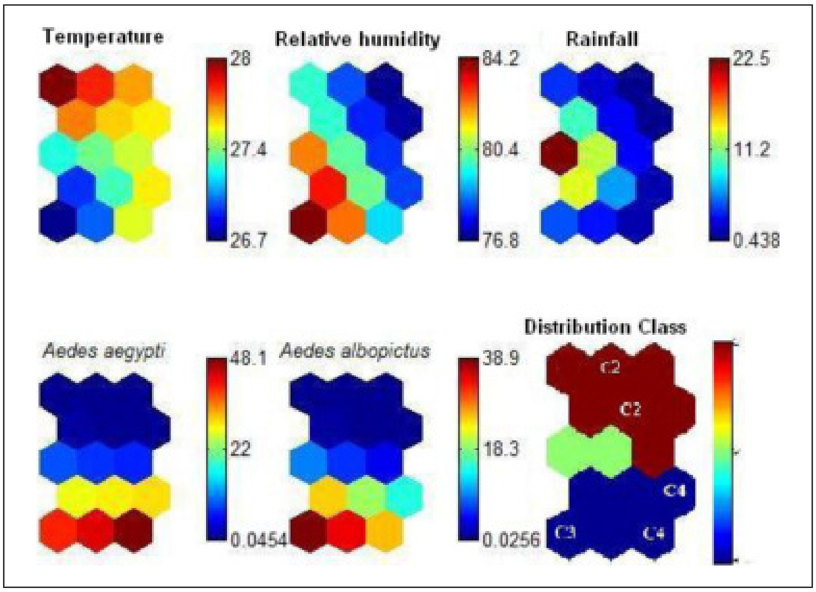

Figure 2. Relationship between physical parameters and distribution classes (SOM, Clustering and Analysis). Colored bars on the right of component plans indicate the scale (high and low) of physical parameters (temperature, relative humidity and rainfall) and the *Aedes* species. Colored bar for distribution class (C2 and C4) indicates distance of data and weighted vectors. Red: high; yellow: moderate; blue: low.

According to Becker *et al.* (2003), *Aedes* has become successful in adapting to environments especially with high population densities. Low population density in the rural areas (except for Kampung Durian Burung), may result in the absence of breeding sites. Hence, reduce the distribution of *Aedes* population. The absence of breeding sites of *Aedes* sp. in Kampung Cina might be due to good hygienic practices among the residence. Hygiene plays an important role in reducing distribution of mosquito population (Vythilingam *et al.*, 2008). Kampung Cina is among major tourist area in the city of Kuala Terengganu and the City Council (MBKT) carried out a campaign to clean-up the entire Kampung Cina area and thus reduced the rubbish such as used plastic bottles and other possible breeding containers.

In urban areas, the most dominant species is *Ae. aegypti* compared to *Ae. albopictus* and this is in agreement with research conducted by Hemme *et al.* (2009) who concluded that *Ae. aegypti* has successfully adapted to the urban environment. In rural areas *Ae. albopictus* is the dominant species and it was suggested that *Ae. albopictus* is currently the most dominant species in Malaysia (Foley *et al.*, 2007). This species has successfully adapted to semi-urban environment and rural areas due to their ability to adapt in a variety of habitats. *Aedes albopictus* are always found outside houses and this species can breed either in artificial or natural containers. Thus it can be concluded that population distribution of *Ae. albopictus* was higher in rural areas because rural areas may provide more suitable breeding sites for *Ae. albopictus*. Our result also shows that both *Ae. aegypti* and *Ae. albopictus* shared many breeding sites. Factors that may lead to the partnership are temperature, humidity, pH value and the percentage of dissolved oxygen, while the two species also are derived from the same genus, allowing the selection of features in same breeding site (Hribar *et al.*, 2004). However, Hribar *et al.* (2004) asserts that the two species is not dependent and do not affect each other.

Factors such as clear and stagnant water bodies are able to attract *Aedes* sp. to breed because of the high oxygen content in the water bodies. Other factor, for example, humidity also plays an important role in the distribution of mosquito populations (Becker *et al.*, 2003). Malaysia generally has a relatively high humidity due to dry and wet seasons occurring throughout the year. This will encourage the *Aedes* sp. to breed because high humidity will result in increment of *Aedes* sp. distribution. The rainy season will also increase the percentage of humidity and this will increase the rate of breeding mosquitoes in the area (Ranjeeta *et al.*, 2008). Rainy season will also increase the number of breeding sites because of the existence of many stagnant water bodies around the areas that are affected by the rainfall. Some parts in Malaysia will receive a high rate of rainfall during certain time each year, for example during the monsoon season, from November until March, in the East Coast of Peninsular Malaysia (Wong *et al.*, 2009). This will indirectly provides a suitable environment for the *Aedes* sp. to breed. However, continuous rainfall may also reduce the number of mosquito breeding sites due to lack of water reservoir especially in abandoned containers. Heavy rainfall will cause water to flow out from containers and hence will not contribute in creating new breeding sites for *Aedes* sp. After a period of continuous rain, the breeding sites of mosquitoes will increase drastically because of the emergence of stagnant water reservoir in abandoned containers. *Aedes* sp. prefer to breed in moderate temperature. The moderate temperature is very important for feeding behavior of adult mosquitoes and mosquito larvae growth rate (Romi *et al.*, 2006). According to the study conducted by Hemme *et al.* (2009), the suitable temperature for *Aedes* sp. to breed is between 26°C to 32°C. Temperature plays an important role in determining the size of the mosquitoes that will grow into an adult and also the nutritional content as well as their genetic factors (Alto *et al.*, 2008; Kamimura *et al.*, 2002).

Results of our study showed that urban areas provided more breeding habitats for *Ae. aegypti* compared to rural areas. In urban and rural areas, frequent distribution (class C4) of *Ae. aegypti* and *Ae. albopictus* was recorded respectively. This frequent distribution was recorded when the humidity was high while both temperature and rainfall were low. Since the number of dengue cases are rising each year, it is hoped that the information reported in this study would help to increase awareness among citizens to keep the environment around their premises clean in order to reduce numbers of mosquitoes and their breeding habitats and eventually reduce the number of dengue cases especially in Malaysia.

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REFERENCES

Alto, B.W., Reiskind, M.H., Lounibos, L.P. (2008). Size alters susceptibility of vectors to dengue virus and dissemination. *American Journal of Tropical Medicine and Hygiene* **79**: 688-695.

Apiwathnasorn, C., Asavanich, A., Komalamisra, N., Samung, Y., Prummongkol, S. & Kanjanopas, K. (2006). The relationship between the abundance of mansonia mosquitoes inhabiting a pest swamp forest and remotely sensed data. *Southeast Asian Journal Tropical Medical Public Health* **37**: 34-38.

Becker, N., Petric, D., Zgomba, M., Boase, C., Dahl, C., Lane, J. & Kaiser, A. (2003). *Mosquitoes and Their Control*. New York: Kluwer Academic/Plenum Publishers: 1-3, 169-174, 213-226.

Chen, *et al.*, Lee., H.L., Stell-Wong, S.P., Lau, K.W. & Sofian-Azirun, M. (2007). Container survey of mosquito breeding sites in a university campus in Kuala Lumpur, Malaysia. *Dengue Bulletin* **33**:187-193.

Dieng, H., Rahman, G.M.S., Abu Hassan, A., Salmah, M.C., Aziz, A.T., Satho, T., Miake, F., Zairi, J., Sazaly, A. & Morales, R.E. (2012). Unusual developing sites of dengue vectors and potential epidemiological implications. *Asian Pacific Journal of Tropical Biomedicine* **2**: 228-232.

Dzieczkowski, A. (1972). Badania ilociowe olimakow buczyn *po*³udniowo-zachodniej Polski [Quantitative researches of the beech malacofauna in south-west Poland]. Studium ekologiczno faunistyczne. *Prace Komisji Biologicznej PTPN* **35**: 243-332.

Foley, D.H., Rueda, L.M. & Wilkinson, B.C. (2007). Insight into global biogeography from country speciesrecords. *Journal of Medical Entomology* **44**: 554-567.

Hemme, R.R., Tank, J.L., Chadee, D.D. & Severson, D.W. (2009). Environmental conditions in water storage drums and influences on *Aedes aegypti* in Trinidad, West Indies. *Acta Tropica* **112**: 59-66.

Horsfall, W.R. (1955). "*Mosquitoes. Their Bionomics and Relation to Disease.*" Ronald Press, New York.

Hribar, L.J., Vlach, J.J., Demay, D., James, S.S., Fahey, J.S. & Fussell, E.M. (2004). Mosquito larvae (culicidae) and other diptera associated with containers, storm drains and seawage treatment plants in the Florida Keys, Monroe County, Florida. *Florida Entomologist* **87**: 199-203.

Kamimura, K., Matsuse, I.T., Takahashi, H., Komukai, J., Fukuda, T., Suzuki, K., Aratani, M., Shira, Y. & Mogi, M. (2002). Effect of temperature on the development of *Aedes aegypti* and *Aedes albopictus*. *Medical Entomology and Zoology* **53**: 53-58.

Kementerian Kesihatan Malaysia. (2015). Situasi semasa demam denggi di Malaysia. Retrieved from http://www.moh.gov.my/index.php/database_stores/store_view/17 (4 Oktober 2015).

Kwa, B.H. (2008). Environmental change, development and vector borne disease: Malaysia's experience with filariasis, scrub typhus and dengue. *Environmental Development Sustainable* **10**: 209-217.

Mandal, S.K., Ghosh, A., Bhattacharjee, I. & Chandra, G. (2008). Biocontrol efficiency of odonate nymphs against larvae of the mosquito, *Culex quinquefasciatus* Say, 1823. *Journal of Acta Tropica* **106**: 109-114.

Muhammad-Aidil, R., Imelda, A., Jeffery, J., Ngui, R., Wan Yusoff, W.S., Aziz, S., Lim, Y.A.L. & Rohela, M. (2015). Distribution of mosquito larvae in various breeding sites in National Zoo Malaysia. *Tropical Biomedicine* **32**: 183-186.

Nazri, C.D., Abu Hassan, A. & Rodziah, I. (2013). Habitat characterization of *Aedes* sp. breeding in urban hotspot area. *Procedia- Social and Behavioural Sciences* **85**: 100-109.

Obenauer, P., Allan, S. & Kaufman, P. (2010). *Aedes albopictus* (Diptera: Culicidae) oviposition response to organic infusions from common flora of suburban Florida. *Vector Ecology* **35**: 301-306.

Ranjeeta, L.M., Sharma, P. & Srivastava, C.N. (2008). Correlation between population dynamics of mosquito larvae and their habitat qualities. *Entomology Research* **38**: 257-262.

Rohani, A., Chan, S.T., Abdullah, A.G., Tanrang, H.I. & Lee, H.L. (2008). Species composition of mosquito fauna in Ranau, Sabah, Malaysia. *Journal of Tropical Biomedicine* **25**(3): 232-236.

Romi, R., Severini, F. & Toma, L. (2006). Cold acclimation and overwintering of female *Aedes albopictus* in Roma. *Journal of American Mosquito Control Association*. **22**(1): 149-151.

Rueda, L.M. (2004). Pictorial keys for the identification of mosquitoes (Diptera: Culicidae) associated with Dengue virus transmission. *Zootaxa* **589**: 1-60.

Russell, R.C. (2004). The relative attractiveness of carbon dioxide and octenol in CDC and EVS-type light traps for sampling the mosquitoes *Aedes aegypti* (L.), *Aedes polynesiensis* Marks, and *Culex quinquefasciatus* Say in Moorea, French Polynesia. *Journal of Vector Ecology* **29**: 309-314.

Saleeza, S.N.R., Norma-Rashid, Y. & Azirun, M.S. (2013). Mosquito species and outdoor breeding places in residential areas in Malaysia. *Southeast Asian J Trop. Med. Public Health* **44**(6): 963-969.

Saifur, R.G., Hassan, A.A., Dieng, H., Salmah, M.R.C., Saad, A.R. & Satho, T. (2013). Temporal and spatial distribution of dengue vector mosquitoes and their habitat pattern in Penang island Malaysia. *Journal of the American Mosquito Control* **29**: 33-43.

Vythilingam, I., Chan, S.T., Shanmugratnam, C., Tanrang, H. & Choi, K.H. (2005). The impact of development and malaria control activities on its vectors in the Kinabatangan area of Sabah, East Malaysia. *Acta Tropica* **96**: 24-30.

Wan-Norafikah, O., Nazni, W A., Noramiza, S., Shafa'Ar Ko'Ohar, Heah. S.K., Nor-Azlina, A.H., Khairul-Asuad, M. & Lee, H.L. (2012). *Sains Malaysiana* **41**: 1309-1313.

Wong, C.L., Venneker, R., Uhlenbrook, S., Jamil, A.B.M. & Zhou, Y. (2009). Variability of rainfall in Peninsular Malaysia. Hydrology & Earth System Sciencs Discussions **6**: 5471–5503.

Zhang, L.Y. & Lei, C.L. (2008). Evaluation of sticky ovitraps for the surveillance of *Aedes (Stegomyia) albopictus* (Skuse) and the screening of oviposition attractants from organic infusions. *Annals of Tropical Medicine & Parasitology* **1202**: 399-407.