POPULATION DYNAMICS OF ADULT MOSQUITOES (DIPTERA: CULICIDAE) IN MALARIA ENDEMIC VILLAGES OF KUALA LIPIS, PAHANG, MALAYSIA

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Abstract. Mosquitoes in malaria endemic areas needs to be monitored constantly in order to detect demographic changes that could affect control measures. A 12-month mosquito population survey was conducted in several malaria endemic areas in Pos Lenjang, Kuala Lipis, Pahang, Malaysia. Collection of mosquitoes using a human landing catch technique was carried out indoors and outdoors for 12 hours from 7:00 PM to 7:00 AM for 42 nights. *Anopheles maculatus* Theobald (31.0%), *Armigeres flavus* Leicester (11.3%), *Armigeres annulitarsis* Leicester (11.0%), *Culex vishnui* Theobald (9.6%) and *Aedes albopictus* Skuse (7.0%) were the predominant species caught in the study area. The salivary gland and midgut of all anopheline mosquitoes were dissected to determine the presence of malaria parasites but none were positive. A high rate of human biting by *An. maculatus* was detected during December, but the rate was lower in January. Mosquito larvae were carried by the rapid current of the river downstream causing a decrease in the larvae population. Of the five predominant species, only *Ar. annulitarsis* exhibited a significant positive correlation in numbers with monthly rainfall ($p<0.05$). *An. maculatus* biting activity peaked during 10:00 PM to 11:00 PM. *Ae. albopictus, Ar. annulitarsis*, and *Ar. flavus* exhibited similar activities which peaked during 7:00 PM to 8.00 PM.

Keywords: adult mosquito, population dynamic, malaria endemic villages, Malaysia

INTRODUCTION

Malaria remains the most significant vector-borne parasitic disease in the tropical and subtropical world (Sungsit *et al*, 2006), including Malaysia, even though malaria control activities over the past few decades have greatly reduced the incidence of malaria (MOH, 1994). Malaria is a disease caused by protozoa and transmitted by the bite of infective mosquitoes (Sadonsham and Thomas, 1965). *Anopheles maculatus* is the principal vector of human malaria in Peninsular Malaysia (Reid, 1968). The Malaria Eradication Program (MEP) was launched in Malaysia in 1969. In 1980, the MEP objectives were modified to a more realistic approach towards controlling the disease, known as the Malaria

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Fig 1–Map of study sites in Pos Lenjang, Kuala Lipis, Pahang, Malaysia.

Control Program (MCP) (Rahman *et al*, 1997). In 1983, the MCP was replaced by the Vector-borne Disease Control Program (VBDCP).

The VBDCP in Malaysia covers activities in prevention and control of vector-borne diseases, such as malaria, dengue, filariasis, typhus, Japanese encephalitis, kala-azar, chikungunya, plague and yellow fever (MOH, 2001). In Peninsular Malaysia, the risk of malaria is high among aborigines living in the interior, in hilly cleared jungles, where they live in bamboo huts with attap roofs, such as in Pos Lenjang, Kuala Lipis and Pahang, where the present study was conducted.

The malaria vector control program carried out in the villages by the Disease Control Division of the Ministry of Health, carries out of indoor residual house spraying with 20 mg/m^2 deltamethrin WP every 6 months and insecticide impregnated bednets. Because these methods primarily target *Anopheles* species, it is essential to have a good knowledge about species composition, nocturnal activity and population dynamics of these mosquitoes in order to better understand disease transmission. Knowledge about the epidemiology of other vector borne diseases is also important (Bhattacharyya *et al*, 1995). We studied the biting cycles of 5 predominant mosquito species in the study areas as an effort to prepare data for the vector borne disease program and determine changes in the vector population density to monitor the impact of control measures.

MATERIALS AND METHODS

The study was carried out in Pos Lenjang, Kuala Lipis, Pahang, Malaysia (101^o 32.874′ E, 4^o 15.527′ N). Pos Lenjang consists of 17 aborigine villages with a total of 256 houses. The coordinates of each village were recorded with a hand-held Garmin's GPSMAP® 60CSx, and the findings were later integrated into a Geographic Information System (GIS) database using ArcView 9.2 software (ESRI, 2006) to develop a map of the villages (Fig 1). The villages were chosen based on the highest incidence rate of malaria cases in peninsular Malaysia reported by the Ministry of Health (MOH, 2006).

Mosquitoes were caught indoors and outdoors using a Human Landing Catching (HLC) technique. The collection was carried out from 7:00 PM to 7:00 AM (12 hours) over a 12-month period. Two houses were randomly selected at each village for the survey. Collections were carried out for a total of 3 nights per village. A team of four collectors was stationed both indoors and outdoors after obtaining informed consent from the occupants. All mosquitoes landing on human subjects were collected using 50 x 19 mm glass vials which were subsequently plugged with cotton wool.

All mosquitoes were identified using the keys of Russell *et al* (1963), and Reid (1968) the following morning and separated by species, village and date. Female *Anopheles* were dissected to determine parity and status of the ovaries. The midguts and salivary glands of parous mosquitoes were examined for malaria parasites using standard dissection techniques (WHO, 1975).

A weather station consisting of an automated rain gauge (Oregon Scientific, USA) was installed at key locations in the study sites. An association between the number of mosquitoes of each species and total rainfall was analyzed by linear regression and correlation coefficient. Comparisons of indoor and outdoor biting activities were analyzed by paired *t*-test but if normality was not observed from the data, then a Mann-Whitney rank-sum test was used (Abu Hassan *et al*, 2001). All data were analyzed using statistics software (SPSS 11.5).

RESULTS

A total of 1,435 adult mosquitoes was caught during the survey. The monthly species composition is shown in Table 1. A total of 36 species belonging to 6 genera were identified.

The following anopheline species were found: *Anopheles maculatus* and *An. donaldi*; the following culicine species were found: *Culex bitaeniorhynchus*, *Cx. gelidus*, *Cx. nigropunctatus*, *Cx. pseudosinensis*, *Cx. pseudovishnui*, *Cx. quinquefasciatus*, *Cx. tritaeniorhynchus*, *Cx. vishnui*, *Aedes albopictus*, *Ae. chrysolineatus*, *Ae. desmotes*, *Ae. niveus*, *Ae. poicillius*, *Ae. pseudalbopictus*, *Ae. vexans*, *Armigeres annulitarsis*, *Ar. balteatus*, *Ar. bhayungi*, *Ar. confusus*, *Ar. dentatus*, *Ar. digitatus*, *Ar. dolichocephalus*, *Ar. flavus*, *Ar. inchoatus*, *Ar. joloensis*, *Ar. longipalpis*, *Ar. magnus*, *Ar. mahadevani*, *Ar. pectinatus*, *Ar. pendulus*, *Ar. subalbatus*, *Ar. traubi*, *Heizmania* sp and *M. dives*.

An. maculatus Theobald (31.0%), *Ar. flavus* Leicester (11.3%), *Ar. annulitarsis* Leicester (11.0%), *Cx. vishnui* Theobald (9.6%) and *Ae. albopictus* Skuse (7.0%) were the predominant species caught in the study area. The other 31 species composed ≤5% of the total. Interaction between total rainfall and the 5 predominant mosquito species caught was determined (Fig 2). Only the total number of *Ar. annulitarsis* exhibited positive linear relationship with monthly rainfall (r^2 = 0.809, p<0.05).

An. maculatus was the most common anopheline species collected. The total number of *An. maculatus* collected per trip outdoor and indoor is shown in Fig 3. The numbers of *An. maculatus* collected outdoors was higher than indoors. All female *Anopheles* mosquitoes were dissected for malaria parasites, but none were positive. The mean parous rate was 41.6%.

Table 1
Total number of mosquitoes caught from July 2007 to August 2008 in Pos Lenjang, Pahang, Malaysia.

Species	Year								Total
	2007			2008					
	July	Sept	Dec	Jan	March	May	June	Aug	
Ae. albopictus Skuse	8	14	28	6	0	22	20	3	101
Ae. chrysolineatus Theobald	3	4	5	2	1	1	3	0	19
Ae. desmotes Giles	0	0	0	0	1	0	1	0	2
Ae. niveus Eichwald	14	0	19	9	3	13	0	0	58
Ae. poicilius Theobald	0	1	0	0	0	0	0	0	1
Ae. pseudalbopictus Borel	11	0	9	1	1	17	1	1	41
Ae. vexans Meigen	0	0	0	0	0	0	0	1	1
An. donaldi Reid	*0*	0	0	0	0	1	0	0	1
An. maculatus Theobald	47	69	85	30	51	78	42	43	445
Ar. annulitarsis Leicester	4	4	84	6	23	34	3	0	149
Ar. balteatus Macdonald	7	4	12	1	3	9	3	0	39
Ar. bhayungi Thurman	1	0	0	1	0	1	0	0	3
Ar. confusus Edwards	0	0	0	0	0	2	0	0	2
Ar. dentatus Barraud	0	5	18	2	1	2	0	0	28
Ar. digitatus Edwards	0	0	3	2	5	1	0	0	11
Ar. dolichocephalus Leicester	4	7	13	6	1	12	2	0	45
Ar. flavus Leicester	25	9	49	14	1	23	33	8	162
Ar. inchoatus Barraud	3	0	1	1	0	3	3	0	11
Ar. joloensis Ludlow	3	0	0	0	0	5	0	0	8
Ar. longipalpis Leicester	2	8	7	0	1	2	0	0	20
Ar. magnus Theobald	0	2	7	2	2	5	3	0	21
Ar. omissus Edwards	0	0	0	2	2	0	0	0	4
Ar. pectinatus/vimoli Edwards	0	0	0	1	0	1	0	0	2
Ar. pendulus Edwards	0	0	8	0	0	1	0	0	9
Ar. subalbatus Coquillett	0	13	4	0	0	3	0	0	20
Ar. traubi Macdonald	0	0	10	0	7	1	0	0	18
Cx. bitaeniorhynchus Giles	0	0	0	0	0	1	0	1	2
Cx. gelidus Theobald	0	2	0	0	2	0	8	0	12
Cx. nigropunctatus Edwards	1	0	0	0	0	0	0	0	1
Cx. pseudosinensis Colless	0	0	0	0	0	1	0	0	1
Cx. pseudovishnui Colless	0	0	0	0	0	3	2	1	6
Cx. quinquefasciatus Say	8	3	0	0	1	2	3	1	18
Cx. tritaeniorhynchus Giles	0	1	0	0	0	0	0	0	1
Cx. vishnui Theobald	0	0	1	0	27	61	39	9	137
Heizmania sp Ludlow	0	0	3	0	0	0	0	0	3
M. dives Schiner	4	4	3	2	3	7	0	1	24

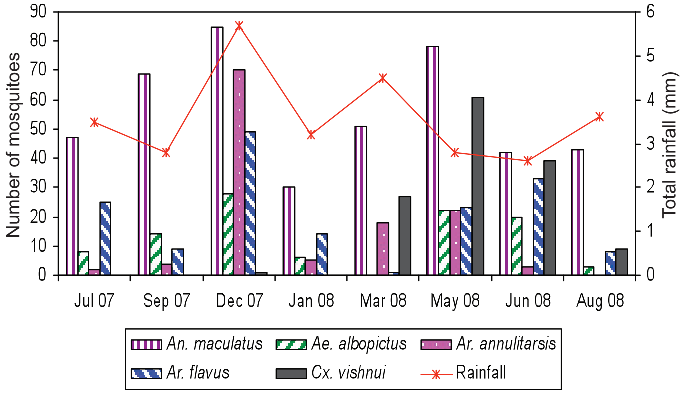

Fig 2–Correlation between total numbers of adult mosquitoes collected per trip and total rainfall in Pos Lenjang, Pahang, Malaysia.

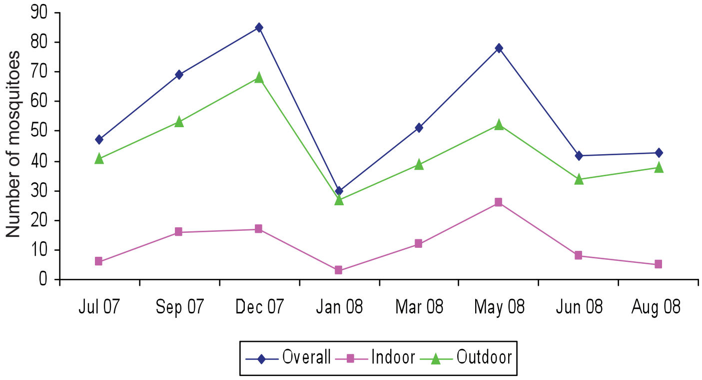

Fig 3–Total number of *An. maculatus* collected indoors and outdoors per trip in Pos Lenjang, Pahang, Malaysia.

The indoor and outdoor biting activities of *An. maculatus* are shown in Fig 4. The highest peak of indoor biting activity was observed at 12:00 AM, while outdoor biting peaks were observed at 11:00 PM, 1:00 AM and 5:00 AM. Outdoor biting activity was higher than indoor biting activity towards the end of the night ($p<0.05$).

The outdoor biting activities *of Ar. flavus* (Fig 5), *Ar. annulitarsis* (Fig 6) and *Ae. albopictus* (Fig 7) had a similar trend but the indoor/outdoor ratios were not significantly different ($p>0.05$). The feeding activity of *Ar. flavus* started immediately at 7:00 PM with the highest peak occurring at 8:00 PM (Fig 5). *Ar. flavus* showed almost no biting activity after 10:00 PM both indoors and outdoors and no significant differences between indoor and outdoor biting activity were observed ($p>0.05$). *Ar. annulitarsis* fed largely during the first two hours of the night (Fig 6). *Ar. annulitarsis* was still present after midnight but in smaller numbers. A small peak was observed in outdoor biting activity at 12:00 AM and 5:00 AM. Although the indoor and outdoor biting activity of *Ar. annulitarsis* was erratic after 10:00 PM, no significant difference was observed ($p>0.05$).

The indoor and outdoor biting activity of *Ae. albopictus* (Fig 7) was significantly different ($p<0.05$). The peak occurred immediately after sunset for both indoor and outdoor biting activity. Outdoor biting activity was lower for *Ae. albopictus* after 9.00 PM, with several small irregular peaks at 1:00 AM and 5:00 AM. The highest peak for indoor biting activity was observed at 8:00 PM.

Fig 8 shows regarding outdoor biting activity, *Cx. vishnui* was present throughout the night with the highest peak from 12:00 AM until 3:00 AM, and another small peak at 5:00 AM. *Cx. vishnui* indoor biting activity was highest at 9:00 PM, followed by 11:00 AM, 3:00 AM, 4:00 AM and 6:00 AM. More *Cx. vishnui* were caught indoors than outdoors from 7:00 PM to 9:00 PM and 6:00 AM. Significant differences were observed between indoor and outdoor

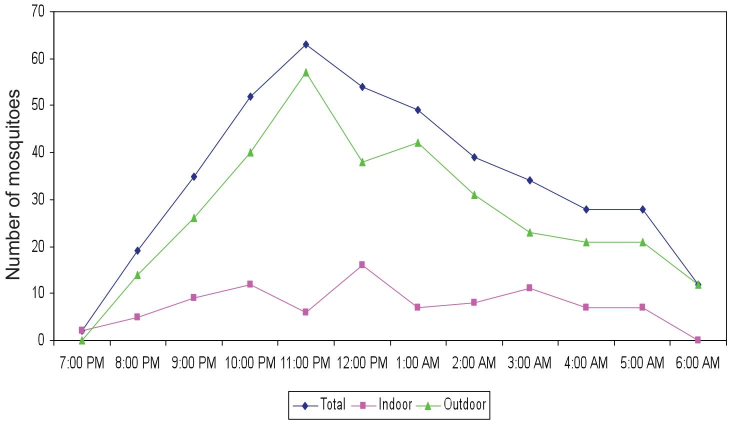

Fig 4–Biting activity of *An. maculatus* in Pos Lenjang, Pahang, Malaysia.

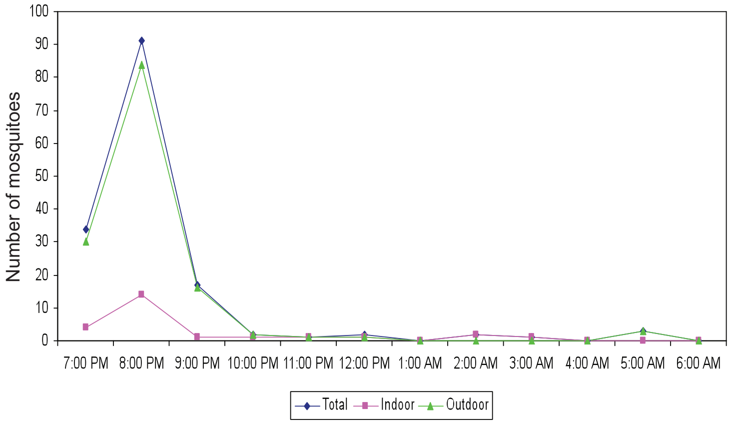

Fig 5–Biting activity of *Ar. flavus* in Pos Lenjang, Pahang, Malaysia.

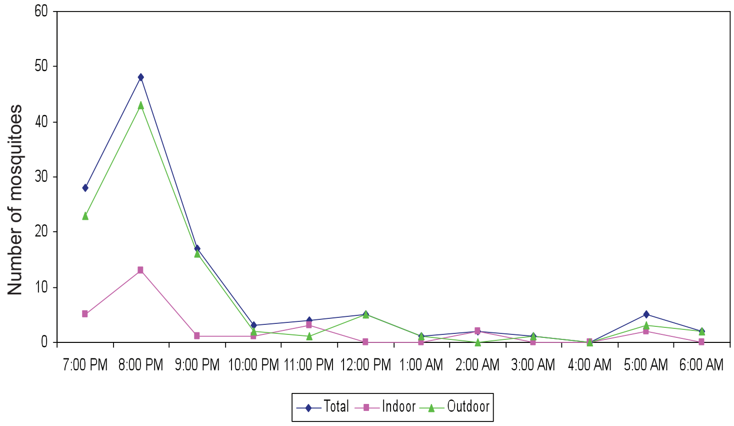

Fig 6–Biting activity of *Ar. annulitarsis* in Pos Lenjang, Pahang, Malaysia.

biting activity with *Cx. vishnui* (p<0.05).

DISCUSSION

Rohani *et al* (2006) determined biting is the most important activity examined in order to understand biting cycles of the vector species. Collections of mosquitoes from outdoors and indoors were analyzed to understand biting cycle of vector species. The study area is located in hilly terrain surrounded by secondary forest with most villages being riverine. Although none of the *An. maculatus* specimens dissected was infected with the malaria parasite during this study, Rohani *et al* (2006) reported that *An. maculatus* has been incriminated as a vector of malaria in the study area. *An. maculatus* bit both indoors and outdoors in our study, similar to the findings of Loong *et al* (1988), Abu Hassan *et al* (2001), and Rohani *et al* (2006). A high rate of human biting by *An. maculatus* was detected during December 2007, and May 2008, but the rate of biting was lower during January 2008 in the study area. *An. maculatus* breeds in sunlit or partially shaded seepages, pools formed in streams, edges of ponds, ditches and swamps with much vegetation (Mike, 2000). Extremely heavy rain-

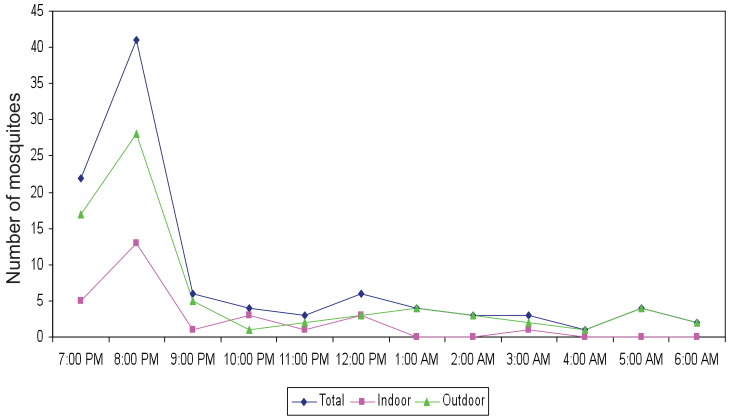

Fig 7–Biting activity of *Ae. albopictus* in Pos Lenjang, Pahang, Malaysia.

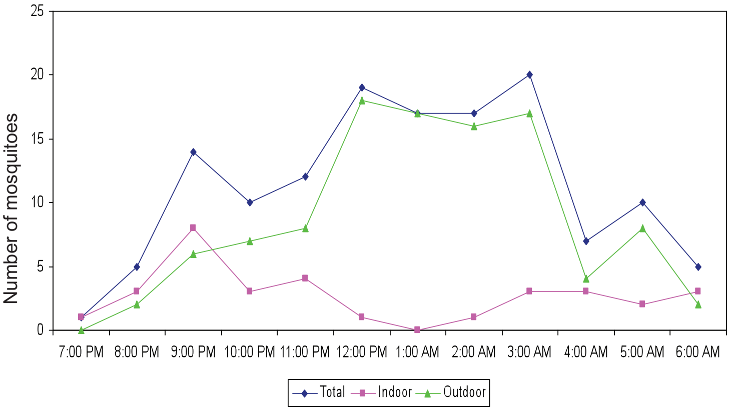

Fig 8–Biting activity of *Cx. vishnui* in Pos Lenjang, Pahang, Malaysia.

fall in December 2007 might have flushed the mosquito larvae out of their breeding sites and decreased the *An. maculatus* population during January 2008, but no significant regression was observed. Sadonsham and Thomas (1965) found *An. maculatus* biting activity peaked at 9:00 PM and midnight. Our study found *An. maculatus* biting extended from 11:00 PM to 1:00 AM. The extended biting activity of the *Anopheles* mosquitoes suggests early use of bed nets may reduce exposure to host-seeking mosquitoes (Abu Hassan *et al*, 2001).

The *Armigeres* subgenus *Leicesteria* is common in the Malaysian forest where bamboos are found. Some species of *Leicesteria* may play a part in the transmission of animal filariasis (Macdonald, 1960). *Armigeres* species comprised 38.7% of the total mosquito numbers during the study period. Of these, *Ar. flavus* (*n*=162) was the most abundant species, followed closely by *Ar. annulitarsis* (*n*=149). *Ar. flavus* is the only species which has been commonly collected from open and accessible breeding places, such as bamboo stumps. *Ar. annulitarsis* normally breeds in young and living bamboo with small holes, usually at heights below 3 meters (Macdonald, 1960). A positive linear regression between total rainfall and *Ar. annulitarsis* species numbers shows heavy rainfall provided extensive breeding habitats and increased *Ar. annulitarsis* populations.

Of the *Aedes* species seen, *Ae. albopictus* was the dominant species in the study area. *Ae. albopictus,* known as the Asian tiger mosquito, is a day biting mosquito and is a vector of dengue in Malaysia. In the forested areas, tree holes and a variety of natural bamboo and rock pools are the breeding sites for this mosquito (Macdonald and Traub, 1960). According to Lo and Narimah (1984), although the *Ae. albopictus* population has been related to rainfall in Asia, including Malaysia, in our study, no significant regression was observed.

Among the *Culex* species collected during the study, *Cx. vishnui* was the predominant species (*n*=137), followed by *Cx. quinquefasciatus* (*n*=18) and *Cx. gelidus* (*n*=12). In India, *Cx. vishnui* is a vector of Japanese encephalitis (JE) (Service, 2000), while in Sepang, Selangor, JE virus has been isolated from *Cx. quinquefasciatus* and Cx. *gelidus* (Vythilingam *et al*, 1997). *Cx. vishnui* breeds in ricefields, ground pools and ditches in plains and foothill areas.

Vythilingam *et al* (1995) and Rohani *et al* (2006) reported that *An. maculatus* biting activity in Kuala Lipis, Pahang peaked from 10:00 PM to 3:00 AM but in our study it peaked from 10:00 PM to 11:00 PM. Four other potential vector species were seen in our study. These entomological data regarding mosquito behavior and patterns of feeding provides important information for vector control programs.

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