DUSK TO DAWN ACTIVITY PATTERNS OF ANOPHELINE MOSQUITOES IN WEST TIMOR AND JAVA, INDONESIA

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Abstract. Malaria is a serious health issue in Indonesia. We investigated the dusk to dawn anopheline mosquito activity patterns, host-seeking and resting locations in coastal plain, hilly and highland areas in West Timor and Java. Adult mosquitoes were captured landing on humans or resting in houses or animal barns. Data analyzed were: mosquito night-time activities; period of peak activity; night-time activity in specific periods of time and for mosquito resting locations. Eleven species were recorded; data were sparse for some species therefore detailed analyses were performed for four species only. In Java *Anopheles vagus* was common, with a bimodal pattern of high activity. In West Timor, its activity peaked around midnight. Other species with peak activity around the middle of the night were *An. barbirostris* and *An. subpictus*. Most species showed no biting and resting preference for indoors or outdoors, although *An. barbirostris* preferred indoors in West Timor, but outdoors in Java. *An. aconitus* and *An. annularis* preferred resting in human dwellings; *An. subpictus* and *An. vagus* preferred resting in animal barns. *An. barbirostris* preferred resting in human dwellings in West Timor and in animal barns in Java. The information is useful for planning the mosquito control aspect of malaria management. For example, where mosquito species have peak activity at night indoors, bednets and indoor residual spraying should reduce malaria risk, but where mosquitoes are most active outdoors, other options may be more effective.

Keywords: Anopheline mosquitoes, overnight activity, biting preferences (landing rates), resting preferences, Java, West Timor, Indonesia

INTRODUCTION

Malaria is a life-threatening disease in Indonesia with an estimated 15 million cases and 42,000 deaths each year (UNDP, 2004; MoH.RI-CDC, unpublished report, 2006; WHO SEARO, 2007). It is transmitted through the bites of infected *Anopheles* spp mosquitoes, of which there are 24 species recorded in Indonesia (O'Connor and Soepanto, 1989; Takken *et al*, 1990; Laihad, 2000). The most important malaria vectors include *An. sundaicus, An. subpictus, An. barbirostris, An. maculatus*, *An. aconitus* and *An. balabacensis* (Takken *et al*, 1990; Harijanto, 2000).

Important factors contributing to malaria transmission include mosquito density and habitat associations, vector competence, dusk to dawn activity patterns

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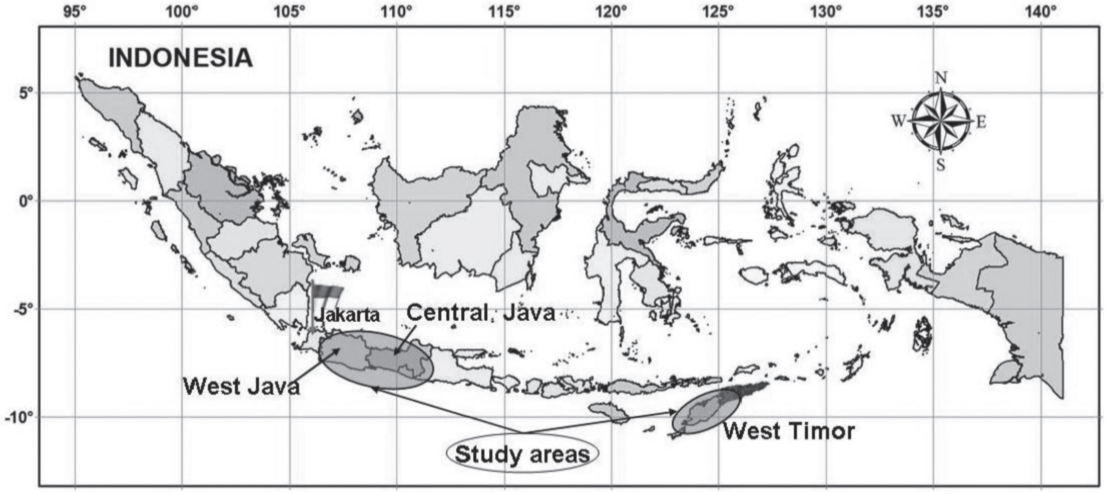

Fig 1–Location of study areas.

and host-seeking/feeding habits (Takken *et al*, 1990). Knowledge of the timing and location of mosquito host-seeking behavior is important for planning appropriate malaria control. For example, using bednets is likely to be effective in areas with species that are known to bite at night and indoors (Pates and Curtis, 2005; Killeen *et al*, 2006).

Important risk factors for malaria that are related to mosquito behavior include feeding, host seeking and resting preferences. These include feeding on humans (anthropophily) or other animals (zoophily) (Warrell and Gilles, 2002); seeking hosts and resting outdoors (exophily), or indoors (endophily) (Warrell and Gilles, 2002).

The subject of the research reported here focuses on anopheline mosquito activity patterns in West Timor and Java between dusk and dawn and with respect to host-seeking and resting locations, at the household level.

MATERIALS AND METHODS

Study areas

Ten villages (5 in West Timor and 5 in Java) were chosen (Fig 1), representing different types of topography (Ndoen *et al,* 2010). These were coastal plain, hilly, and highland areas. The villages were selected based on their pattern of malaria from a baseline study, the availability of local assistants and access constraints. The villages were located in West Timor, West Java and Central Java (the latter two together referred to as Java). Previous research in the study areas, reported in Ndoen *et al* (2010) showed that some anopheline species had topographic preferences. For example, hilly areas (mainly rice fields) were associated with *Anopheles annularis, An.vagus* and *An. subpictus*, the latter only in Java. In coastal areas the main anophelines included *An. barbirostris, An. maculatus* and *An. subpictus*, the latter only in West Timor.

Adult mosquito surveys

To explore mosquito host-seeking/biting behaviors collections were made every two weeks of adult mosquitoes landing on humans and also resting mosquitoes (Table 1). Landing rates were taken as a proxy measure for biting activity. The methods were based on those

Table 1
Summary of adult landing and resting collections.

Location (village)	Topographic type	No. of collector- nights	Dates of survey
West Timor			
Noelbaki	Coastal	12	21 Jun, 5 Jul 2006
Tablolong	Coastal	12	20 Jul, 18 Aug 2006
Liufeto-Tuadale	Coastal, flat, lakes	18	2 Aug, 31 Aug, 28 Dec 2006
Sikumana	Hilly	36	29 Jun, 13 Jul, 27 Jul, 24 Aug, 12 Sep 2006, 5 Jan 2007
Haumenbaki	Highland	48	25 Jun, 7 Jul, 21 Jul, 3 Aug, 25 Aug, 21 Sep, 19 Oct, 3 Nov, 17 Nov, 30 Nov, 7 Dec, 14 Dec 2006
Total West Timor		126	
Java (West and Central)			
Lodji	Coastal	24	30 Nov, 13 Dec, 28 Dec 2006, 12 Jan 2007
Kertajaya	Coastal	24	1 Dec, 12 Dec, 27 Dec 2006, 9 Jan 2007
Langkap Jaya, Lengkong	Highland	24	27 Nov, 11 Dec, 27 Dec 2006, 12 Jan 2007
Sadang Kulon	Hilly	24	18 Jan, 2 Feb, 17 Feb, 3 Mar, 17 Mar, 31 Mar 2007
Jojogan	Hilly – rice field	24	18 Jan, 02 Feb, 17 Feb, 3 Mar, 17 Mar, 31 Mar 2007
Total Java		120	

recommended by WHO (WHO, 2003a, b) and summarized below.

Adult mosquitoes were caught when landing on the bare legs or hands of human volunteers during the night. Four to six people (depending on the location) were employed to do this. The collectors were divided into indoor and outdoor groups. Both indoor and outdoor catches were carried out throughout the night, generally from 06:00 PM to 06:00 AM, every hour during which landing counts were made for forty minutes, followed by ten minutes collecting mosquitoes resting on the wall in the house, and outdoors in animal shelters. The last ten minutes were used for collectors to rest and change positions. To avoid collector bias, each collector was rotated to a different location on an hourly basis. The collectors were equipped with torches, aspirators and paper cups to catch and store the mosquitoes.

Mosquitoes were identified using the identification key for female Anophelines of Indonesia provided by the Indonesian Ministry of Health (O'Connor and Soepanto, 1989).

Data analysis

Data was treated in its raw form or was converted to a standard measure of Man-Hour Density (MHD), that is the average number of mosquitoes landing per collector per hour, analysed using chi-square analysis.

Comparing West Timor and Java – dusk to dawn activity

Non-parametric statistics were used to analyse the frequency data. A Kolmogorov-

Smirnov two-sample test and chi-square analysis were performed to investigate if the night-time activities varied significantly between West Timor and Java. If they did not differ significantly the data were combined for both areas.

Curve-fitting of Man Hour Density data

Landing rates were partitioned into 12 one-hour periods from sunset to sunrise. To simplify the variations from hour to hour for this analysis, a smoothing routine was used, and a polynomial curve-fitting was chosen as described below:

The 12 points were used as dependent values for a polynomial regression on hour of night, and regressions were computed up to sixth order polynomials –*ie,* seven models were computed, 1) with just the mean, 2) the intercept plus trend on time, 3) the intercept plus trend plus the square of time, 4) plus the cube up to (7) plus the sixth power of time. The seven models thus comprised an estimate of the mean plus an increasing number of polynomial terms. The best model to fit to the data was the highest order model for which the highest order polynomial was significant at $p< 0.05$.

This was a curve-selection procedure for the purpose of smoothing, not a modelling process, and the nature of the fitted curve is not the subject of the computations. As such, the stringencies of polynomial regression analysis were not of importance to the procedure – it was only intended to identify a satisfactory curve to summarize changes in activity through the night.

Once the curve was identified, it was used to estimate the time of night that activity peaked (the peak point on the curve) and the period of the night within which the majority (90%) of activity occurred. That was the cumulative percentage of the total mosquitoes counted from the first landing of the night with time estimated from the curve to the nearest 10 minutes.

Activity by time periods between dusk and dawn

In order to obtain a clearer understanding of the timing of host-seeking activity, a more detailed analysis was done by time periods. The night was divided into five periods as follows:

1) Hourly basis (hour by hour); 2) The six hours before midnight and the six hours after midnight; 3) Evening (three hours after sunset) and Morning (three hours before sunrise); 4) Dusk (the hour after sunset) and dawn (the hour before sunrise); 5) Crepuscular (the first and last hours of the night) and night (10 hours between crepuscular).

Chi-square analysis was carried out on the frequency data to investigate if there was a relationship between host-seeking activity and period of observation.

Indoors and outdoor activity and resting preferences

Chi-square analyses were also used to test for relationships between mosquito species and location of host-seeking activity outdoors (exophily) or indoors (endophily) and resting preferences in human houses or animal barns.

RESULTS

Eleven species of mosquitoes were captured. Of these, there were very few of four species (*An. flavirostris, An. indefinitus, An. kochi,* and *An. tesselatus* and these were only caught in the resting observations); these species were not further analyzed. Table 2 shows the total number of mosquitoes caught in the landing counts for the remaining seven species

Table 2
Landing counts of mosquitoes in West Timor and Java: the most important malaria vectors are shown in bold (This does not include resting counts: see Table 6.)

Place	***An. aconitus***	*An. annularis*	***An. barbirostris***	*An. maculatus*	***An. subpictus***	***An. sundaicus***	*An. vagus*	Total
W Timor	4	3	179	2	693	0	107	988
Java	175	2	82	57	3	20	84	423
Total	179	5	261	59	696	20	191	1,411

Table 3
Kolmogorov-Smirnov two-sample test for night-time activity patterns using landing count data.

Species	Max diff	*p*	Pattern	Further analysis
An. aconitus	0.487	0.300	Similar	Combined
An. barbirostris	0.138	0.220	Similar	Combined
An. subpictus	0.935	0.011	Different, but very little data from Java	Combined
An. vagus	0.495	0.000	Different	Kept separate

in West Timor and Java. Of these, four species (shown in bold) are considered to be especially important malaria vectors (Takken *et al*, 1990). Altogether a total of 1,411 individuals were caught, of which 70% were caught in West Timor, with all species present. They were predominantly (70%) *An. subpictus* (an important malaria vector); less than 1% comprised of *An. maculatus*, *An. annularis* and *An. sundaicus* and these numbers were too small to analyse reliably. In Java *An. aconitus* comprised 40% of the total and *An. barbirostris* and *An. vagus* each accounted for just less than 20% of the total.

Dusk to dawn activity pattern for both areas

The results of the Kolmogorov-Smirnov two-sample test are shown in Table 3.

Table 3 shows that *An. aconitus* (p = 0.300) and *An. barbirostris* (p = 0.220) had similar night activity patterns and so data from West Timor and Java were combined. *An. subpictus* (p = 0.011) night-time activity differed between West Timor and Java but, due to the very small number in Java (3 out of 696 mosquitoes) the Java data was not further analysed. *An. vagus* was the only *Anopheles* species in this study that showed a significantly different night-time activity pattern between Java and West Timor (p = 0.000), so the data for this species was kept separate for both areas.

Fig 2 summarizes the best-fit curves for the activity times for the four species and Table 4 summarizes the activity by the five periods (1-5) as defined above. For *An. aconitus* 90% of catches were before 04:20 AM. The period of greater activity

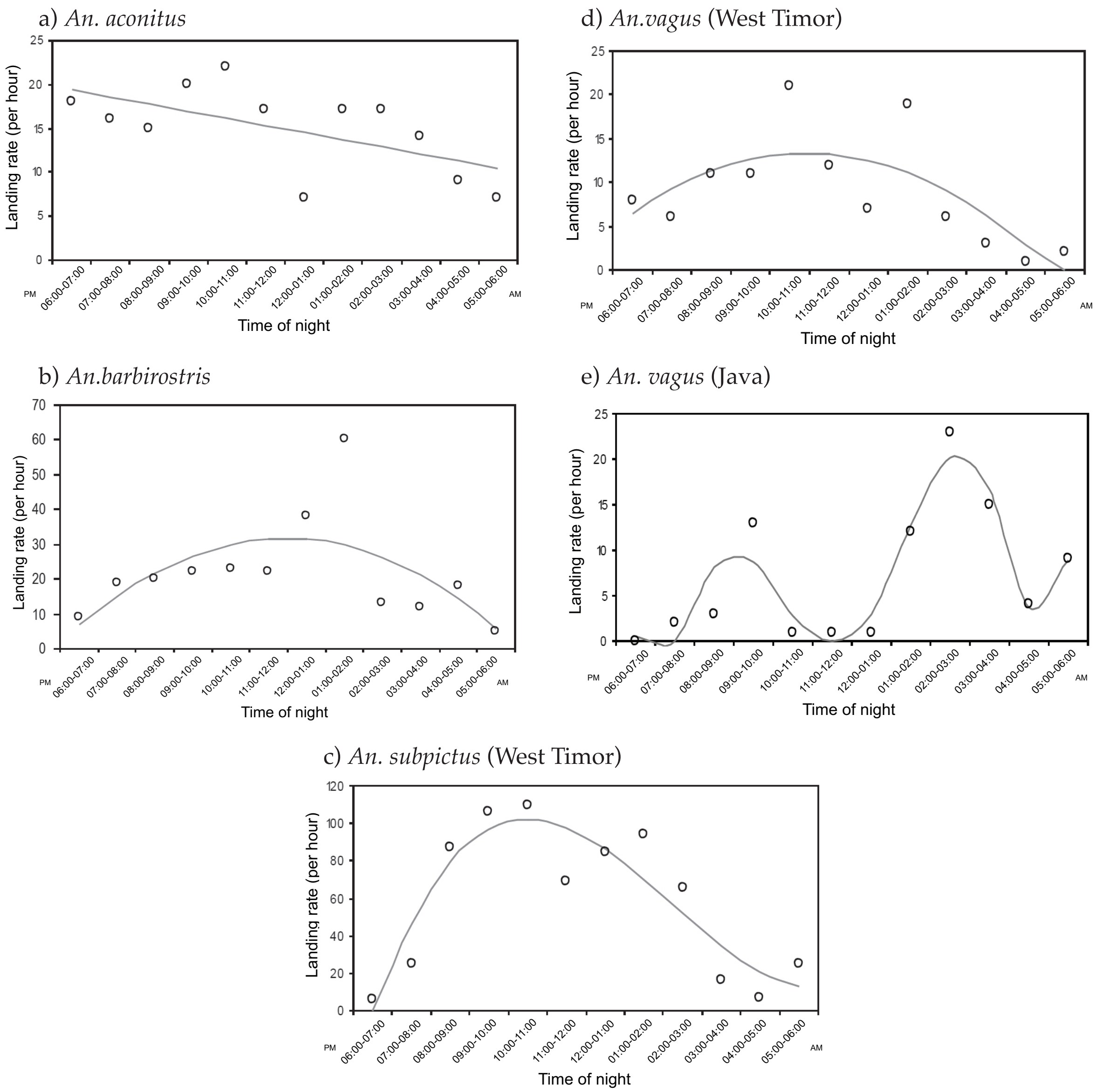

Fig 2–Dusk to dawn host-seeking activity of *Anopheles* spp in West Timor and Java.

for *An. aconitus* (Fig 2a) was early in the night ($p = 0.0057$), with significantly more specimens collected at dusk than at dawn (p=0.033), evening than morning and before rather than after midnight (p=0.028). *An. barbirostris* (Fig 2b) was more active during the night than in the crepuscular hours (p = 0.000). Its activity peaked around midnight, with 90% being caught between 07:20 PM and 04:30 AM. For *An. subpictus* (Fig 2c), 90% of activity occurred between 19:40 PM and 04:00 AM. It had significantly higher activity in the evening than in the morning, with a rapid increase after sunset (p=0.000), before midnight rather than after (p=0.000), during the night rather than in the crepuscular hours (p=0.000), and there was significantly

Table 4
Chi-square analysis results of dusk to dawn activity of *Anopheles* spp (significant results are in bold).

Species	Location J- Java WT – West Timor	Comparisons between 2 periods overnight									
		1.Hour-by-hour (each of 12 hours, significance relates to hour by hour differences)		2. Before or after midnight (6 hrs *vs* 6 hrs)		3. Evening or morning (1 hr evening *vs* 1 hr dawn)		4. Dusk or dawn (3 hrs after sunset *vs* 3 hrs before dawn)		5. Crepuscular or night 2 hrs 06:00 PM, 06:00 AM *vs* 10 hrs overnight)	
		Time of greater activity	p	Time of greater activity	p	Time of greater activity	p	Time of greater activity	p	Time of greater activity	p
An. aconitus	J and WT	No	0.094	**Before**	**0.0057**	**Evening**	0.033	**Dusk**	**0.028**	No	0.094
An. barbirostris	J and WT	**Yes**	**3.1×10^{-18}**	No	0.055	No	0.15	No	0.290	**Night**	**9.6×10^{-7}**
An. subpictus	J and WT	**Yes**	**1.9×10^{-57}**	**Before**	**3.1×10^{-5}**	**Evening**	**5.5×10^{-8}**	**Dawn**	**6.4×10^{-4}**	**Night**	**5.4×10^{-18}**
An. vagus	**J**	**Yes**	**1.9×10^{-13}**	**After**	**1.6×10^{-6}**	**Morning**	**6.2×10^{-5}**	**Dawn**	**0.0027[a]**	No	0.14
An. vagus	**WT**	**Yes**	**1.1×10^{-6}**	**Before**	**0.0027**	**Evening**	**0.00064**	No	**0.058[b]**	**Night**	**0.042**

[a]data were sparse and the test may have low power
[b]data are sparse, but numbers suggest greater activity at dusk than dawn

Table 5
Summary results of total mosquitoes from human landing collections indoors (In) and outdoors (Out) in West Timor and Java.

Species	Location	West Timor		Java		Preference for indoor or outdoor			
		In	Out	In	Out	$\chi 2$	df	p	Preference
An. aconitus	Java only	Insufficient data		48	127	2.84	1	0.09	No preference
An. barbirostris	West Timor	79	100	0	0	40	1	1.9×10^{-4}	Indoor
	Java	0	0	1	81	287	1	2.4×10^{-64}	Outdoor
An. subpictus	West Timor	190	503	Insufficient data		2.82	1	0.09	No preference
An. vagus	West Timor	28	79	0	0	0.93	1	0.34	No preference
	Java	0	0	24	60	1.92	1	0.17	No preference

Table 6
Summary results of total *Anopheles* spp resting places in Java and West Timor (significant results in bold).

Species	Location Java/West Timor	West Timor Total numbers		Java Total numbers		Resting preference			
		Animal	Human	Animal	Human	χ^2	df	p	Preference
An. aconitus	Java and West Timor	6	0	889	36	0.55	1	0.46	No difference
An. annularis	Java and West Timor	9	4	476	11	2.22	1	0.14	No difference
An. barbirostris	West Timor	6	62			**128**	1	**1.3×10^{-29}**	**Human**
	Java			434	2	**2.42**	1	**0.12**	**Animal**
An. subpictus	West Timor	259	113			0.34	1	0.56	No difference
	Java			411	1	3.6	1	0.06	No difference
An. vagus	West Timor	171	4			60.6	1	**6.8×10^{-15}**	**Animal**
	Java			2,327	13	10.2	1	**1.4×10^{-03}**	**Animal**
**An. indefinitus*	Java			481	0	Only found in animal barns			
Total numbers		451	83	5,018	63				

more activity just before dawn than just after sunset (p=0.000). In West Timor, *An. vagus* (Fig 2d) was already active at sunset and activity peaked at 11:00 PM with 90% of activity between 06:50 PM and 03:30 AM. In contrast, in Java, *An. vagus* (Fig 2e) commenced activity well after sunset and peaked after midnight (around 02:40 AM) with 90% of activity occurring between 08:30 PM and 05:30 AM.

Indoors or outdoors host-seeking

Table 5 shows the results from the analyses of the location of human landing collection surveys in West Timor and Java (indoors or outdoors). Only *An. barbirostris* showed any significant preference, with a tendency for indoor host seeking in West Timor and outdoor host seeking in Java.

***Anopheles* resting preference**

Table 6 summarizes the resting collection data. Most species were associated with animal barns (84% in West Timor and 99% in Java). In West Timor, only *An. barbirostris* were collected in larger numbers in houses than in barns (62 out of 68).

DISCUSSION

According to Takken *et al* (1990) the Indonesian malaria eradication program from 1955 to 1969 failed because it focused on the vector and not its ecology. Effective malaria management needs effective mosquito control, which requires understanding of vector behavior. A major outcome of the research reported here is information on the vectors' behavioral ecology that is important to malaria risk: time of host-seeking, location of host-seeking and resting places. This study provides information that could be used to increase the effectiveness of the malaria vector control program, so that control efforts can be specifically targeted against

Table 7
Summary of host-seeking and resting data (most significant vectors shown in bold).

Species	Peak time of host seeking	Preference		Comment
		Host seeking location (indoor/outdoor)	Resting location (house or animal barn)	Potential strategy
An. aconitus	Early evening declining through the night	No preference	No preference, but tended to rest in houses	Use of personal protection recommended. As the adults appear to be ubiquitous, targeting the larval habitats, if known, may be an option. Indoor residual spraying (IRS) may also be an option.
An. barbirostris (West Timor)	Peak at midnight	(Indoor)	Endophilic (House)	Bednets and IRS.
An. barbirostris (Java)	Peak at midnight	(Outdoor)	Exophilic (Animal barn)	Personal protection: long clothing, avoiding outdoors. May be lower priority as it appears to be exophilic.
An. subpictus	Peak during night	No preference	No preference	Bednets, personal protection.
An. vagus (West Timor)	Peak during the night	No preference	Animal barn	Lower priority in West Timor as appeared to be zoophilic.
An. vagus (Java)	Bi modal peak	No preference	Animal barn(zoophilic)	Lower priority in Java as appeared to be zoophilic.

the local vector species.

To synthesise the results, Table 7 provides a broad overview, together with some indicative suggestions for management. The greatest risk for malaria transmission is where mosquito host seeking behavior coincides with the places and times of human presence. Thus indoor host seeking at night is a risk factor. In this study, *An. barbirostris* was found indoors in West Timor with peak host-seeking for the hour around midnight. *An. barbirostris* has been confirmed as a malaria vector in many parts of Indonesia (Lien *et al*, 1977; Lee *et al*, 1983; Mardiana, 1990; Boesri, 1994; Boewono *et al*, 1997; Maguire *et al*, 2005). The species was found outdoors in Java and this is consistent with a study in Central Sulawesi, where *An. barbirostris* was more active outside human dwellings with peak feeding hours between 11:00 PM and 04:00 AM (Garjito *et al*, 2004).

When species are most active overnight but have no preference for indoor or outdoor locations, the risk may be spatially distributed. In this research, that includes risk associated with *An. aconitus, An. subpictus* and *An. vagus* (West Timor). However, *An. aconitus* may be a significant risk as it was active overnight from 06:00 PM onwards. This contrasts with the findings of Rijadi (2002) for this species in Central Java, who reported peak biting hours for *An. aconitus* in the evening between 06:00 PM and 07:00 PM. *An. subpictus* has also been confirmed as an important malaria vector (Lee *et al*, 1983; Mardiana, 1990). *An. subpictus* in West Timor had no preference for indoors or outdoors or for resting inside houses or animal barns; however, this species poses a significant risk due to its long night-time host-seeking activity. Mardiana (1990) and Barodji (2000) reported that *An. subpictus* was active both indoors and outdoors, with peak activity hours from 01:00 AM to 02:00 AM. Previous studies over several years have confirmed that *An. vagus* is one of the main malaria vectors in West Timor (CDC-NTT, 2005). Different behavioral patterns between *An. vagus* from West Timor and Java were observed; such variation within a species in different locations was also reported by Pates and Curtis (2005) and this may indicate that there needs to be different management strategies appropriate to each place. This is relevant to integrated vector management programs.

Although *An. annularis* and *An. maculatus* have been reported as present in Indonesia, very few were caught in this study and neither species is considered to be a significant malaria vector. It was not surprising that very few *An. annularis* were caught in the human landing collections, as Noerhadi (1960) noted that it was one of the most difficult *Anopheles* species to find; this species has not been yet confirmed as a malaria vector (Boesri, 1994; Ompusunggu *et al*, 1994) thus this species may not be a significant malaria threat and hence a lower priority in vector control programs. *An. maculatus* showed no preference to feed indoors or outdoors although Sandosham and Thomas (1983) reported that it was zoophilic and preferred to feed in open areas; no clear night activity pattern was observed. In contrast, other studies have reported that *An. maculatus* is more active outdoors, especially in highland areas with peak activity hours between 09:00 PM to 03:00 AM (Lestari, 1990). Such knowledge of a species' behavior in particular areas is important for planning control and monitoring the outcome of control measures.

The results of this study have enhanced the value of earlier research for the same areas that identified the topographic preferences of the anopheline mosquitoes

(Ndoen *et al*, 2010). Where there are clear topographic preferences, knowing also the diurnal activity patterns may help focus control to both the locations and times that increase risk of exposure to host-seeking anophelines. Thus *An. vagus* and *An. subpictus* (Java), both associated with hilly rice fields, have been identified in the current research as having night activity patterns. The coastal species of particular concern includes *An. barbirostris* (indoor and with night time activity) and *An. subpictus* in West Timor, where it showed a very small (not significant) preference for resting in animal shelters.

In conclusion, this research has shown that mosquito behavior between dusk and dawn can be species specific and can vary within a species by location. Understanding this can potentially improve malaria prevention and mosquito control activities, by focusing the type of method (*eg*, bednets, spraying, yard sanitation) on the important vectors and at the times and places of their activity. Knowledge of mosquito resting places can help determine appropriate prevention and control measures so that people can avoid being exposed to host seeking mosquitoes.

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