



## Factors associated with distribution of *Anopheles sundaicus* in coastal area, Kuala Penyu, Sabah

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### Abstract

Malaria is still one of the most important diseases in Malaysia. A study was conducted in the coastal area of Kuala Penyu, Sabah, where the malaria vector, *Anopheles sundaicus* s.s is highly abundant. This study aims to determine the biting patterns and the relationship between meteorological factors and the abundance of *An. sundaicus* s.s. Mosquitoes were collected indoors and outdoors by human landing collection from 1800 to 0600 h. All collected mosquitoes were identified to the species level. Meteorological parameters including monthly temperature, rainfall, relative humidity and wind speed were analyzed. A total of 2,294 *An. sundaicus* s.s were collected (2,010 outdoor and 284 indoor). Peak biting time for outdoor occurred between 2100 to 2200 h and between 2400 to 0100 h for indoor. The seasonal abundance of *An. sundaicus* s.s appeared to be influenced by monthly temperature patterns ( $p=0.0044$ ), compared to other meteorological parameters. The population densities of *An. sundaicus* s.s showed the greatest abundance during high temperature season, April to August with the highest density was recorded in May, while December was the lowest. This study provides information on biting patterns throughout the night and seasonal abundance of *An. sundaicus* s.s. This information serves as a basis for providing health education on the importance of personal protective equipment among residents to prevent mosquito bites, as well as to improve vector control strategy planning to be more efficient, effective and reduce operating costs.

**Keywords:** *Anopheles sundaicus*, biting time, malaria vector, meteorology, seasonal abundance

### 1. Introduction

Malaria is a major public health problem, with an estimated 207 million cases and 627,000 deaths worldwide in 2012. There were 3.4 billion people at risk of malaria in 2012, 1.2 billion of these were at high risk (>1 case per 1000 population), mostly living in the Africa Region (47%) and the South-East Asia Region (37%) [43]. More than half of the world's population live on the island and coastal areas and the number is expected to increase 54% by 2050 compared to 2000 [40]. Malaria burden in island and coastal areas has also contributed to high number of malaria cases due to the presence of malaria vectors in the area. Transmission of disease still occurs in some coastal foci where it is a fatal disease that is endemic in poor rural areas [39]. Therefore, growing numbers of people will be placed at risk by an increase in malaria vector population in coastal areas [27].

Malaria is transmitted by biting of the female anopheline mosquito species. Out of 75 Anopheles species recorded in Malaysia, the nine species viz., *An. balabacensis*, *An. maculatus*, *An. campestris*, *An. sundaicus*, *An. letifer*, *An. donaldi*, *An. cracens*, *An. latens*, and *An. flavirostris* are vectors of malaria [26]. *An. sundaicus* is a major malaria vector on islands and coastal areas of Southeast Asia [2, 9]. It is widely distributed from northern east India to southern Vietnam, including Myanmar, Thailand, Cambodia, Peninsular Malaysia, and the islands of Nicobar, Andaman, Borneo, and Indonesia [5, 34]. It is considered as either a major or secondary malaria vector depending on region and country it was found [20, 30]. *An. sundaicus* is a dominant malaria vector in the coastal areas of Indonesia viz., Sebatik Island [12], Central Java [3], Southwest Sumba [24], South Sulawesi [6] and Bali [31]. In India, it presently plays an important role in malaria transmission in coastal areas of the

Andaman and Nicobar Islands [1]. *An. sundaicus* is described as an efficient vector in coastal areas of Thailand [25], Malaysia [5], Vietnam [23], and considered a secondary vector in Cambodia and Myanmar [20].

*An. sundaicus* can breed in both brackish water and freshwater [2, 22]. Larvae have been collected in brackish water at many locations [13, 15, 19] and in freshwater near Miri in Sarawak, Malaysia Borneo [5, 18], South Tanapuli in Sumatra, Indonesia [34], and Car Nicobar Island, India [7]. Particularly favorable habitats are coastal fish/shrimp ponds or irrigated inland sea-water canals, but immature stages also inhabit rock pools, mangrove, ponds and swamps [10, 11, 17]. Only a few freshwater sites have been documented across its distribution [5, 34].

Currently, malaria is still one of the most important vector-borne diseases in Malaysia, primarily in Malaysian Borneo (Sarawak and Sabah states), despite showing reduction of cases for a decade. In 2012, there were 4,725 cases which is 63% reduction compared to cases in 2000 (12,705 cases). There has also been a reduction in the number of malaria deaths from 35 in 2000 to 16 deaths in 2012 [21]. Vector control remains the most effective measure to prevent malaria transmission. Understanding mosquito behavior, population dynamics, and the risk of transmission are necessary for the design and evaluation of effective control and preventive measures against malaria. Entomological studies are required to provide evidence-based and practical solutions to clearly define control-oriented research questions. Any malaria control strategy should be based on a thorough understanding of the transmission of the disease including the epidemiological variables, the behavior of the human host, the parasite, and the environment. One of the main causes of malaria control failure is the lack of

understanding of the ecology of vector species. The success of vector control programs is highly dependent on a thorough knowledge of behavior and bionomics of the vector, which must be precisely identified to ensure the application of appropriate control measure [38].

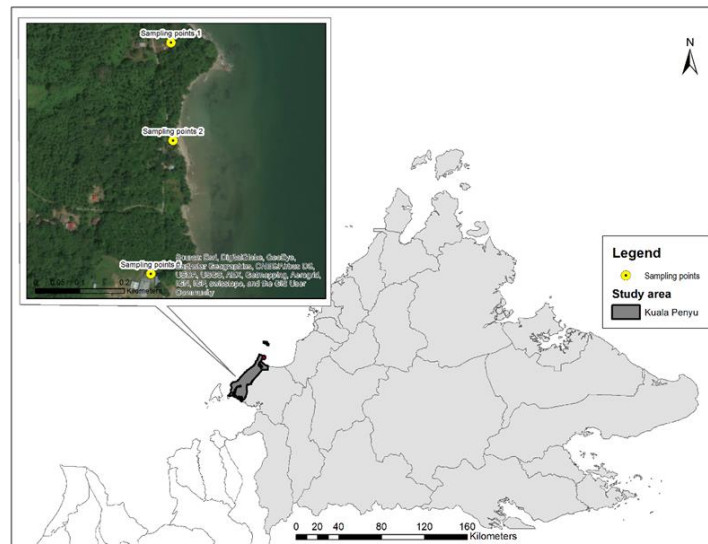
Kuala Penyu is a district located in Sabah state, Malaysia Borneo. It has a coastline of 80 km long and covered with mangrove swamp forest. The ecology of Kuala Penyu provides a suitable habitat for brackish water breeder, *An. sondaicus* s.s which is predominant in the Borneo Island [17]. Malaria cases are also reported periodically with a total of 92 cases recorded from 2000 until 2012 [41]. In addition, Pulau Tiga (Tiga Island), located about 16 km from Kuala Penyu is a major tourist destination. Visitors usually get there by boat from Kuala Penyu, which will be at risk of getting malaria infection. Recently, there is not much information gathered on peak biting time for *An. sondaicus* specifically in Sabah. Knowledge of the biting habits of mosquitoes, especially of the disease vectors, is necessary to plan vector avoidance and control strategies. Furthermore, detailed information on biting activity of vectors during the night hours in different months could help in choosing personal protection measures that would prevent human-mosquito contact and to improve malaria vector control strategies. These studies point to the seasonal and temporal

variation in mosquito biting rhythms. We undertook this study from January to December 2012 with two main objectives: (i) to determine the biting patterns of *An. sondaicus* during different phases of night and months; and (ii) to determine the relationship between meteorological factors and the abundance of *An. sondaicus*.

## 2. Materials and methods

### 2.1 Study area

Kampung Kiaru located in the northern part of Kuala Penyu and west coast area of Sabah; Coordinates: N5°36'42.1" E115°37'12.5" (Figure 1) was chosen as the location of mosquito collection. Three sampling points at which collectors were stationed around the study area were selected. The distance between each sampling points is about 250 meters. Geographical information shows that the location is a coastal area and slightly hilly. Ecological environment consists of palm trees, mangroves, bushes, coconut trees, rubber and oil palms. There are many potential breeding places for *An. sondaicus* in the study area such as streams, mangroves, fish pond, shrimp pond and ditches. Houses in this area are made of brick and wood, as well as having holes and openings that allow mosquito entry and exit. Predominant activities in the area include fishing, shrimp farming, agriculture and cultivation.



**Fig 1:** Kampung Kiaru, Kuala Penyu, Sabah

### 2.2 Mosquito collections

Mosquitoes were collected indoors and outdoors by human landing-biting catches for two nights on the second week of each month from January to December 2012. Captures were performed by four individuals (2 groups of 2 people) per sampling points. The first group was seated 10 meters outside a house while the second group was seated inside a house. Collections were carried out from 1800 h to 0600 h. A pair of collectors worked between 1800 h and 2400 h and a second pair between 2400 h and 0600 h. A one-hour landing-biting collection consisted of 50 minutes of collection and 10 minutes rest. Mosquitoes that landed and tried to bite the bare legs of the baits were collected. At the end of each one-hour collection cycle, mosquitoes collected in vials were placed in a paper cup covered with netting, labeled with the time of collection and the site [42]. Mosquitoes were identified to species using the taxonomy

keys [28].

### 2.3 Meteorological data

Meteorological data of 2012 were obtained from the Institute of Climate Change, Universiti Kebangsaan Malaysia, Selangor. Meteorological parameters included monthly mean, minimum and maximum temperature (°C), rainfall (mm), relative humidity (%) and wind speed (knot).

### 2.4 Statistical analysis

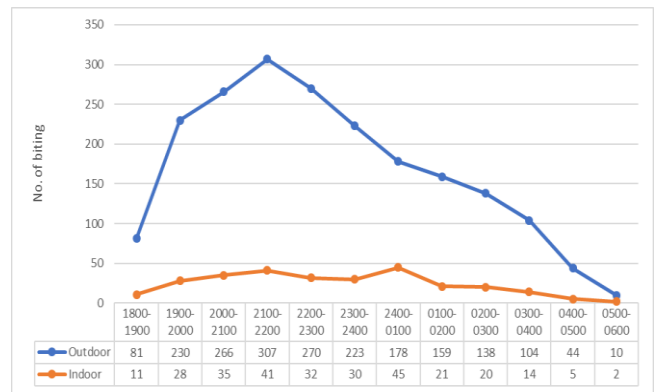
We used Stata version 12 (Stata Corp) for data analysis. Descriptive analysis was done to describe the numbers of biting by hours in a day and months. We also calculated the mean and standard deviation for the number of biting for indoor and outdoor, and meteorological parameters including monthly temperatures, monthly relative humidity, monthly wind speed, and monthly precipitation. We further

tested for mean difference between number of biting in outdoor and indoor. For continuous variables, a Pearson correlation coefficient was computed to assess the relationship between the number of biting and various meteorological parameters. To examine association between total number of biting and various meteorological parameters, we performed linear regression analysis for unadjusted and adjusted that is significant at p-value < 0.05.

**3. Results**

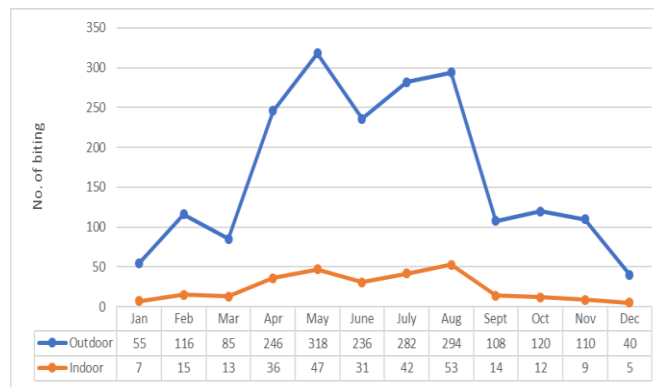
Over a 12-month period, a total of 2,294 *An. sudaicus* s.s was captured with the mean of 191.2 (SD=116.8). Overall higher biting was observed outdoors (87.6%) than indoors (12.4%), with the outdoor/indoor ratios ranging from 5.0 to 8.8. The mean of outdoor-biting was significantly higher than the mean of indoor-biting, p-value < 0.001 (Table 1). *An. sudaicus* s.s was found biting as early as 1800 h to 1900 h with 81 (4.0%) mosquitoes caught outdoors and 11 (3.9%) mosquitoes caught indoors (Figure 2). The highest frequency of outdoor-biting was recorded between 2100 h to 2200 h with 307 mosquitoes (15.3%), while the lowest catch between 0500 h to 0600 h with 10 mosquitoes (0.5%). The highest frequency of indoor-biting was recorded between 2400 h to 0100 h with 45 mosquitoes (15.8%), while the lowest catch rate was between 0500 h to 0600 h with 2 mosquitoes (0.7%). The outdoor-biting pattern shows that

the biting number increased sharply between 1900 h (230 mosquitoes) to 2200 h (307 mosquitoes). Then, the number of bites dropped after 2200 h until 0600 h.



**Fig 2:** Biting times of *An. sudaicus* s.s in Kampung Kiaru, Kuala Penyu, Sabah

Over the past 12 months, the highest density of *An. sudaicus* s.s was recorded in May with a total catch of 365 mosquitoes (15.9%), while December was the lowest with total catch of 45 mosquitoes (2.0%). Abundance of *An. sudaicus* s.s was occurred between April and August 2012 (Figure 3).



**Fig 3:** *An. sudaicus* s.s collected from January to December 2012

**Meteorological parameters**

Table 1 shows variation of meteorological parameters for the year 2012 in the area of study sites. The 12-month mean for temperature was 30.4 °C (SD=0.86), and 201.83 mm

(SD=47.77) for the rainfall in year 2012. The yearly mean for relative humidity was 84.74 % (SD=1.10) while the yearly mean wind speed was 5.75 knots (SD=1.36).

**Table 1:** Variation of meteorological parameters and biting frequency

Month	Number of biting (outdoor)	Number of biting (indoor)	Total Biting	Monthly Mean Temp (°C)	Relative Humidity (%)	Monthly Mean Wind Speed (knot)	Monthly Mean Rainfall (mm)
Jan	55	7	62	29.7	84.91	8.0	146.1
Feb	116	15	131	30.3	85.79	7.0	119.7
Mar	85	13	98	30.9	84.52	8.0	156.5
Apr	246	36	282	30.7	86.02	7.0	152.5
May	318	47	365	31.6	83.33	5.0	216.1
June	236	31	267	31.6	83.33	5.0	213.5
July	282	42	324	30.6	85.21	5.0	207.6
Aug	294	53	347	31.3	83.06	5.0	255.2
Sept	108	14	122	29.0	84.06	5.0	228.4
Oct	120	12	132	29.9	84.72	5.0	277.3
Nov	110	9	119	29.5	86.22	4.0	232.1
Dec	40	5	45	29.7	85.66	5.0	216.9
Mean	167.5	23.67	191.17	30.39	84.74	5.75	201.83
SD	100.04	17.07	116.77	0.86	1.10	1.36	47.77

Notes: All continuous data fulfilled normality assumption. Hence, we reported mean (SD)

Pearson correlation analysis shows only temperature was positively correlated with the number of biting ( $r=0.761$ ,  $p=0.0044$ ), while other meteorological variables such as relative humidity, wind speed and rainfall were not significantly correlated with the number of biting (Table 2). To determine associated factors for the number of biting time and meteorological parameters, results from the multiple linear regression analysis shows that there was a

significant linear relationship between the number of biting and temperature (Table 3). For every 1-unit increment of temperature, the number of biting is expected to increase by about 103 biting (adjusted  $\beta=103.15$ , 95% CI=15.41, 190.89,  $p\text{-value}=0.027$ ), holding other meteorological variables constant. Other meteorological variables, such as relative humidity, wind speed and rainfall did not show any significant linear associations with number of biting.

**Table 2:** Pearson Correlation coefficient between number of biting with meteorological parameters

Parameter	Number of biting	Mean Rainfall (mm)	Relative Humidity (%)	Monthly Mean Temp (°C)	Monthly Mean Wind Speed (knot)
Number of biting	1.00				
Monthly Mean Rainfall (mm)	0.223	1.00			
Relative Humidity (%)	-0.490	-0.403	1.00		
Monthly Mean Temp (°C)	0.761**	-0.050	-0.555	1.00	
Monthly Mean Wind Speed (knot)	0.222	-0.836**	0.187	0.055	1.00

Notes: \*\*Significant at  $p\text{-value} < 0.05$

**Table 3:** Linear regression analysis for association between number of biting and meteorological parameters

Parameter	Simple linear regression		Multiple Linear regression	
	Unadjusted $\beta$	p-value	Adjusted $\beta$ (95% CI)	p-value
Monthly Mean Temperature, °C	103.61 (41.28, 165.95)	0.004	103.15 (15.41, 190.89)**	0.027
Monthly Mean Rainfall (ml)	0.54 (-1.14, 2.22)	0.487	-0.32 (-2.96, 2.31)	0.780
Relative Humidity (%)	-51.94 (-177.09, 13.19)	0.106	-4.09 (-82.20, 74.02)	0.905
Monthly Mean Wind Speed, knot	-26.35 (-84.07, 31.38)	0.333	-38.83 (-121.04, 43.38)	0.301

Notes: \*\*Statistically significant at  $p\text{-value} < 0.05$ , Coefficient of determination ( $R^2$ ) = 0.7033. Model assumptions are fulfilled. No multicollinearity detected.

#### 4. Discussion

In terms of *An. sundaicus* feeding behavior, a wide range of behavioral patterns seem to occur, including indoor-biting (endophagy) and outdoor-biting (exophagy), with varying degrees and predilections for feeding on either human or other animals. *An. sundaicus* behavior is different depending on the locality and their species complex [10]. At least four sibling species are recognized in the complex, *An. sundaicus* species A (*An. epiroticus*), *An. sundaicus* s.s., *An. sundaicus* species D, and *An. sundaicus* species E [2, 9]. In this study, *An. sundaicus* s.s engaged in both endophagy and exophagy, but had a greater outdoor biting frequency. These findings are similar to previous studies conducted in West Java [32], Sebatik Island North Kalimantan [12], and Sumatera, Indonesia [16], where the biting behavior is more prevalent outdoors than indoors.

Our study found that, the biting activity of *An. sundaicus* s.s started from 1800 h and increased from 1900 to 2200 h. The peak biting time outdoors occurred earlier between 2100 to 2200 h, while indoors was a bit later between 2400 to 0100 h. Our findings were further supported by a study conducted in Car Nicobar Island where similar biting times were observed with *An. sundaicus* species D [14]. Other studies have shown contradicting biting times by *An. sundaicus* species A in Chang Island, Thailand where the peak biting time indoors occurred later between 0100 to 0200 h, while peak biting time outdoors occurred earlier between 2000 to 2100 and still occurred between 0100 to 0200 h [29]. In Rayong Province, Thailand, the biting activity occurred throughout the night with the highest peak between 2300 to 2400 h [36]. The biting patterns in Lampung, Sumatera, showed that *An. sundaicus* species A appeared after sundown and continued until sunrise, with peak biting time occurring between 2400 to 0300 h [35].

From observation throughout the year, we recognized the

beginning of *An. sundaicus* s.s biting time was consistent from 1800 h. During this period, most of the residents would be still doing outdoor activities such as recreation, gardening and fishing. Therefore, application of suitable repellents and proper clothing can provide protection against vectors. Mosquito coils, aerosols and repellents can be used effectively to prevent host-seeking mosquitoes in the early parts of the night before the residents go to sleep. Educating residents to improve their knowledge and awareness of personal protection measures by the use of mosquito nets may be an effective measure in reducing the chances of man-mosquito contact while sleeping. The observation that the majority of *An. sundaicus* s.s were collected outdoors, has implications in the control of malaria epidemics because it may limit the effectiveness of Indoor Residual Spraying (IRS). Based on our study, IRS should remain a part of an epidemic control strategy because 14% of *An. sundaicus* s.s were collected indoors. Epidemic malaria control programs that include IRS may need to be supplement with larval control and space spraying around settlements to achieve affective control of an outbreak [33].

The seasonal abundance of *An. sundaicus* s.s in this study appeared to be influenced by several factors, notably monthly temperature patterns. The population densities of *An. sundaicus* s.s showed the greatest abundance during high temperature season (April to August). *An. sundaicus* s.s was found to be active in every collection period throughout the years, with peak densities observed in May and August. In Chang Island, Thailand, observations over two years showed that the greatest abundance occurred during the high temperature season (November to April) with the highest densities in November [29]. *An. sundaicus* seasonal density in South Sulawesi, Indonesia was unpredictable, while malaria transmission was reported to be perennial with a strong peak biting developing in February and March and another in

June and July [6]. Mosquitoes have an optimal range of temperature for survival and development and this closely matches the climate where each vector species is found. Changes in ambient temperature will tend to affect daily probability of survival of the mosquitoes [27]. Temperature affects the development of mosquitoes. At higher temperatures, the gonotrophic cycle which, the physiological process consisting of blood digestion and ovarian development will decrease. The elevation of 1.6°C in ambient temperature would signify an acceleration of mosquitoes' development by 2.3 days [4]. This mean that both gonotrophic cycle would be shortened and the number of mosquito generations would increase. A reduction in the duration of the gonotrophic cycle would make the vectors bite more frequently, thus increasing the probability of transmission of malaria [8].

Meteorological parameters often considered for their impact on mosquitoes including temperature, rainfall, and humidity, but others such as atmospheric particle pollution, sea levels, and wind can also have an impact [27]. However, there were no associations between relative humidity, rainfall and wind speed with *An. sundaicus* s.s density in this study, but the population density tends to increase during wet season. During the rainy season, the number of available habitats is significantly greater than the dry season. Rainfall forms surface pools of fresh water that can provide breeding sites for immature stages [32]. However, excessive rainfall can flush away larvae and eggs and reduce the numbers of breeding sites thereby temporarily lowering the density of mosquitoes. A previous study in Rayong Province, Thailand, reported that *An. sundaicus* species A was found active throughout the year with the highest density in the rainy season [36]. In contrast, the highest density of *An. sundaicus* was reported during the dry season with a slight amount of rainfall in Chang Island [29], Lampung, Sumatera [35] and West Java [32]. During the dry season, lagoons and brackish water impoundments became more suitable habitats for *An. sundaicus* as water flow was impeded from entering the sea, creating large stagnant bodies of water and abundant floating algal mats [37]. Coastal areas, depending on their humidity, are likely to be affected similarly to inland areas by rainfall. However, an additional consideration in coastal areas is that a drier climate can favor the salinity-tolerant vector of *An. Sundaicus* [27].

Although malaria vectors have been identified in Kuala Penyu, not much information about their biology and behavior (population dynamics, biting time, host preference and seasonal abundance) have been described. As only three sites were observed, this does not preclude other potential vector species that playing a greater role in malaria transmission. Thus, continued investigations of the bionomics of the species is needed to determine the appropriateness and effectiveness of ongoing malaria control programs in Kuala Penyu and alternative interventions to reduce the risk of malaria transmission. Further investigations in other localities will better define and map vector distribution in relation to malaria transmission. Moreover, a far better understanding of the full range and preferred *An. sundaicus* s.s larval habitats is also required and a pre-requisite to any meaningful attempt to control this species and transmission of malaria.

## 5. Conclusion

This vector survey provides valuable information on *An.*

*sundaicus* s.s and its potential importance in malaria transmission in Kuala Penyu. Locations where this species found in Kuala Penyu should be targeted for control efforts during malaria epidemics. A proper planning schedule for routine vector control should be implemented so that the activities will be conducted at the time where the vectors become active for biting. Seasonal abundance patterns including the influence of meteorological parameters should be considered in the control strategy planning, so that the outcomes will be more efficient, effective that could reduce operating costs. These findings are important for malaria management program to facilitate in designing malaria vector control programs relevant to local characteristics including the vectors and their activity patterns.

## 6. Acknowledgments

We would like to thank the Director General of Health Malaysia for his permission to publish this article. We also extend our sincere gratitude to the staff of Beaufort and Kuala Penyu Health Office and Institute of Climate Change, Universiti Kebangsaan Malaysia for their indefatigable efforts to facilitate and assist in this study.

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