SOME ENTOMOLOGICAL OBSERVATIONS ON TEMPORAL AND SPATIAL DISTRIBUTION OF MALARIA VECTORS IN THREE VILLAGES IN NORTHWESTERN THAILAND USING A GEOGRAPHIC INFORMATION SYSTEM

Ratana Sithiprasasna [1], Kenneth J Linthicum[2], Gang-Jun Liu[3], James W Jones[1] and Pratap Singhasivanon[4]

[1]Department of Entomology, US Army Medical Component, Armed Forces Research Institute of Medical Sciences, Thailand; [2]Vector-Borne Disease Section, Infectious Diseases Branch, California Department of Health Services, Ontario, California, USA; [3]Department of Geospatial Science, Faculty of Applied Science, RMIT University, Melbourne, Victoria, Australia; [4]Department of Tropical Hygiene, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

Abstract. This spatial and temporal heterogeneity in the distribution of *Anopheles* mosquitos were studied during August 2001 to December 2002 in three villages Ban Khun Huay, Ban Pa Dae, and Ban Tham Seau, in northwestern Thailand in Mae Sot district, Tak Province. The three Karen villages are located about 20 km east of the city of Mae Sot near the Myanmar border. Twenty-one species were collected on human collections during 68 nights of 17 months. *Anopheles minimus* comprised of 86% of the specimens biting man. *An. minimus* was implicated as a vector based on the detection of sporozoite infections using enzyme-linked immunosorbent assays for *Plasmodium falciparum* and *P. vivax*. Seasonal comparison of vectorial capacity and entomological inoculation rate was calculated. *An. dirus* was rarely encountered and probably played little part in transmission in these three villages during the period of study. Information is provided on nightly biting activity, parity rate, infectivity, and adult and larval bionomics. Spatial and temporal comparisons among the collections were displayed on different satellite images including the Normalized Difference Vegetation Index data from on the National Oceanographic and Atmospheric Administration satellites (NOAA/NDVI), the LANDSAT satellite Thematic Mapper (spatial resolution 30x30 m) and the IKONOS satellite (spatial resolution 1x1 m) in a Geographical Information System (GIS).

INTRODUCTION

Although the incidence of malaria in Thailand has been significantly reduced during the past 50 years, in 2002 the annual incidence of malaria-related illness in all of Thailand was 0.82 per 1,000 population with a mortality rate of 0.68/ 100,000 population in 2001 (Fig 1) as assessed by passive case detection (Division of Epidemiology, 2001-2002). Tak Province, the epicenter of multi-drug resistance in Thailand, is situated on the border with Myanmar and consistently exhibits high rates of malaria incidence (3,328 cases/ 100,000 population in 2001).

Thailand is situated at unique zoogeographic crossroads in Southeast Asia and is the home to approximately 13% of the described mosquito species of the world (Harrison, 1980). Tak Province is located in the northern and western mountains in the Oriental Faunal Region (Belkin, 1962), and it has a large number of *Anopheles* species. Epidemiologic and ecologic data on anopheline malaria vectors in northwestern Thailand is complex, related to vegetation distribution, and not well understood (Singhasivanon *et al*, 1999). Information concerning the biology of potential malaria vectors in the region is important in understanding the dynamics of malaria transmission. *Anopheles dirus* Peyton and Harrison is the principal vector of malaria in cen-

Correspondence: Dr Pratap Singhsivanon, Department of Tropical Hygiene, Faculty of Tropical Medicine, Mahidol University, 420/6 Rajvithi Road, Bangkok 10400, Thailand.
Tel: 66 (0) 2644-7483
E-mail: tmpsh@mahidol.ac.th

Table 1
Female anophelines captured during human bait collections made outside houses in Ban Khun Huay, Ban Pa Dae and Ban Tham Seau (August 2001- December 2002).

Species	August 01	September 01	October 01	November 01	December 01	January 02	February 02	March 02	April 02	May 02	June 02	July 02	August 02	September 02	October 02	November 02	December 02	Total
An. aconitus	0	0	9	1	2	0	1	0	0	1	1	2	0	0	0	4	2	23
An. annularis	9	3	0	0	0	0	0	0	0	0	1	7	1	1	3	1	0	26
An. argyropus	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
An. barbirostris	0	12	21	2	0	0	1	0	0	0	0	2	1	2	0	2	3	46
An. campestris	44	171	327	6	36	13	7	1	0	3	23	28	36	35	28	11	6	775
An. culicifacies	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
An. dirus	9	9	48	1	1	0	0	0	0	0	1	8	13	17	5	8	4	124
An. dravidicus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
An. jamesii	0	0	0	3	1	0	0	0	0	0	2	0	0	0	0	0	2	8
An. kochi	8	14	32	0	0	0	0	0	0	0	2	16	3	2	2	4	2	85
An. maculatus	17	15	10	3	8	2	4	1	1	0	22	61	21	18	35	27	56	301
An. minimus	413	1,739	3,355	290	201	251	243	267	90	254	2,302	1,752	627	436	1,159	2,115	168	15,662
An. nivipes	6	4	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	13
An. peditaeniatus	16	116	107	2	0	3	0	0	0	0	0	5	27	21	207	15	1	520
An. philippinensis	1	3	2	0	0	0	0	0	0	0	1	0	0	0	4	2	1	14
An. pseudojamesi	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	4
An. sawadwongporni	3	4	6	5	13	16	10	9	0	1	45	22	2	0	80	53	38	307
An. splendidus	4	0	0	0	0	1	0	0	0	0	2	1	3	1	2	0	0	14
An. tessellatus	25	54	36	0	0	5	0	1	0	0	98	15	4	1	35	18	10	303
An. vagus	0	2	0	0	0	0	0	0	0	0	2	8	4	0	0	0	0	16
An. varuna	1	5	5	2	1	0	0	1	0	0	4	0	3	0	13	1	6	42
Total	560	2,151	3,958	316	263	291	266	280	91	259	2,506	1,931	745	534	1,575	2,263	299	18,288

tral and eastern Thailand (Rosenberg *et al*, 1990), and *An. minimus* Theobald (Rattanarithikul *et al*, 1996) and *An. maculatus* Theobald (Kittayapong *et al*, 1992) have been implicated in malaria transmission in peninsular Thailand.

In this study, we report observations on the seasonal abundance and parity rates of adults, and immature habitats of potential malaria vectors in three villages located in the malaria endemic forests of Tak Province, northwestern Thailand. We also evaluate the role of these anophelines in the transmission of malaria in this area by testing specimens collected for the presence of *Plasmodium falciparum* (Welch) and *P. vivax* (Grassi and Feletti) circumsporozoite (CS) antigen by enzyme-linked immunosorbent assay (ELISA).

The aims of this study were to use geographical information systems (GIS) to : 1) examine the temporal and geographic distribution of man-biting adult *Anopheles* mosquitos to determine whether there is a link between adult mosquito distribution and location of larval habitats, 2) identify larval habitats that produce key vector species, and 3) to incorporate our findings into a decision matrix that will allow us to identify key areas that are critical to maintaining malaria transmission and that would be susceptible to targeted control efforts. It was considered that if we examine the temporal and geographic distribution of human malaria cases among the three villages, we should be able to derive the spatial-temporal predictive model of malaria transmission in this area.

MATERIALS AND METHODS

Study area

Mosquitos were collected in three villages in Mae Sot district, Tak Province (Figs 2-3). Ban Khun Huay, Ban Pa Dae, and Ban Tham Seau are Karen villages, located about 20 km east of the city of Mae Sot near the Myanmar border with Thailand. Each of the villages is located in a deciduous woodland in the eastern watershed of the Moei River, which drains westward into the Salween River. All three villages are situated in a valley between forested hills which rise to elevations of 200 to 400 m. Small intermittent streams that are dry during most of the dry season drain the hills and feed into larger streams that are divided into a network of channels within each village. Ban Khun Huay contains ~ 50 houses and a population of 250; Ban Pa Dae contains ~ 90 houses and a population of 500; and Ban Tham Seau contains ~ 60 houses and a population of 260. Houses are built on stilts, constructed of split bamboo with the front side largely or completely open and thatched with teak leaves. There are few animals in the villages other than chickens, dogs, pigs and a few water buffalos. All three villages are highly endemic for both falciparum and vivax malaria.

Mosquito collections

Mosquito collections were conducted monthly for 4 days each month from August 2001 to December 2002. Human bait collections were made between 18 00 and 06 00 hours by two men sitting beside houses located in the middle of each village. The men working in 2 six-hour shifts which changed at midnight. A rest period was taken during the last 10 minutes of each full hour. At the end of each 1-hour collection cycle, mosquitos collected in vials were placed in a paper cup covered with netting, labeled with the time of collection, and stored alive in cool boxes. Mosquitos were collected on a total of 68 nights during August 2001 to December 2002. All anopheline mosquitos were captured and the time of collection recorded. Mosquitos of other genera were discarded and not considered further in this study. Adult specimens, progeny of some females, and reared immatures were identified using the keys to adult anopheline mosquitos in Thailand (Harrison and Scanlon, 1975; Harrison, 1980; Rattanarithikul and Green, 1986; Rattanarithikul and Panthusiri, 1994). *Anopheles dirus* consists of a species complex that can be distinguished only by cytogenetic or DNA probe techniques. We did not use these techniques in this study. Within the geographical range for the present study, *An. dirus* species A, C, and D have been found (Baimai, 1988). The ovaries of field-collected adults were examined to determine parity status as described by Detinova (1962).

All collections were conducted using a standard methodology to permit temporal and spatial comparisons between collections and statistical evaluation. Hourly temperature and relative hu-

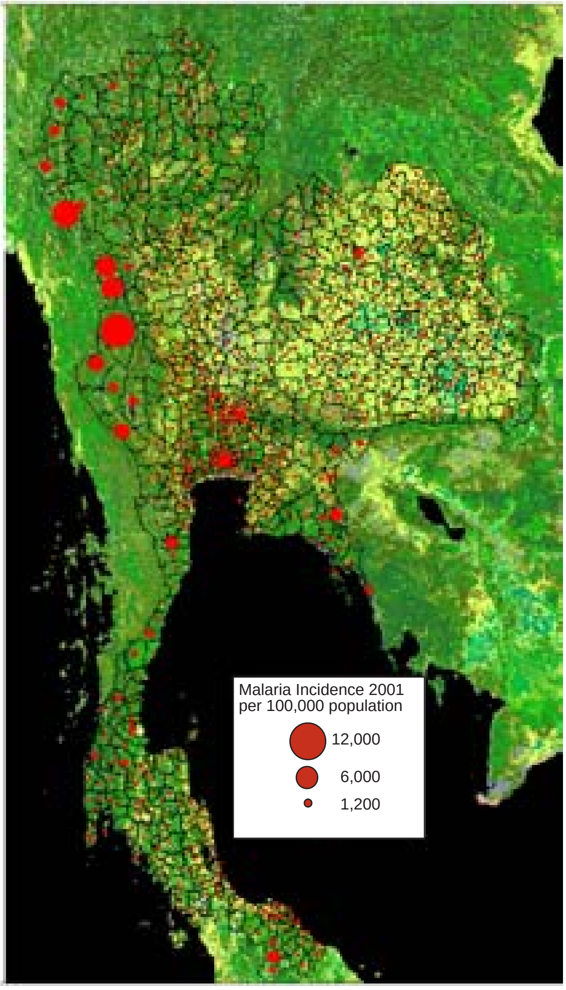

Fig 1–Distribution of malaria incidence per 100,000 population for the year 2001 by district plotted on district boundary layer in a GIS. National Oceanographic and Atmospheric Administration satellite Normalized Difference Vegetation Index (NOAA/NDVI) data images, showing forest cover in dark green color, is used for base map. Water shown in dark black color.

midity and daily rainfall data were collected from each village (Fig 5) using Stowaway Data Loggers (Onset Computer Corporation, Pocasset, MA). Monthly weather data routinely collected from the local district weather station 20 km from the study site was also obtained for comparison from the Climatology Division, Meteorological Department, Ministry of Information and Communication Technology, Bangkok, Thailand.

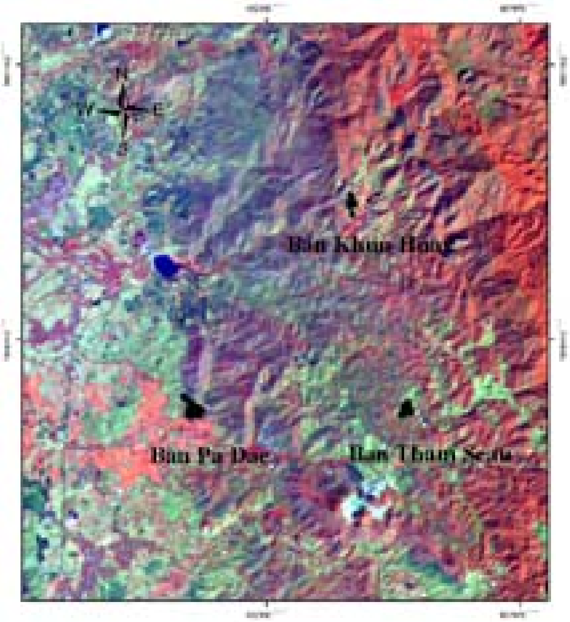

Fig 2–Locations of each house (black dot) in three villages utilizing Global Positioning System data overlaid on LANDSAT 5 Thematic Mapper data (band combination 4, 5, 2).

Enzyme-linked immunosorbent assay (ELISA)

All mosquitos were bisected behind the second pair of legs, triturated in a non-specific protein blocking buffer (1% BSA, 0.5% casein, 0.01% thimerosol, 0.02% phenol red and PBS at pH 7.4) for circumsporozoite (CS) antigen testing. *P. falciparum* and *P. vivax* CS antigens were detected and identified using enzyme-linked immunosorbent assays (ELISA) developed by Wirtz *et al* (1987, 1992) and modifications described in detail by Rattanarithikul *et al* (1996). The head/thorax and abdomen of each mosquito were tested separately by ELISA for the presence of CS protein to *Plasmodium falciparum*, *P. vivax* VK247, and *P. vivax* VK210. A positive ELISA (optical density equal to or greater than twice the lowest positive reference control (25 sporozoites for *P. falciparum*, 25 for VK210, and 50 for VK247, and exceeding the highest negative control) of the head and anterior thoracic segments containing the salivary glands was defined as evidence that a mosquito was infectious. *An. minimus* were tested in pools of five or fewer mosquitos collected at the same time and location.

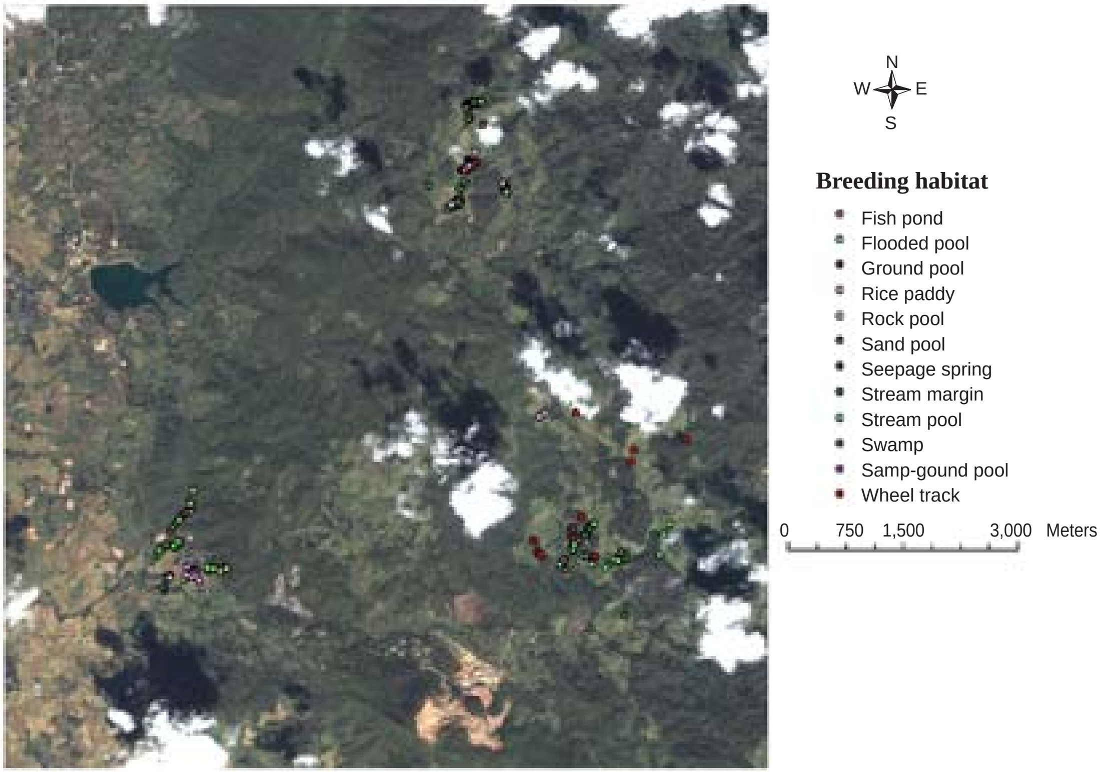

Fig 3–Thematic maps showing location and categories of breeding habitats of malaria mosquitos around the three villages overlaid on IKONOS satellite data (spatial resolution 1x1 meter) displayed in true color.

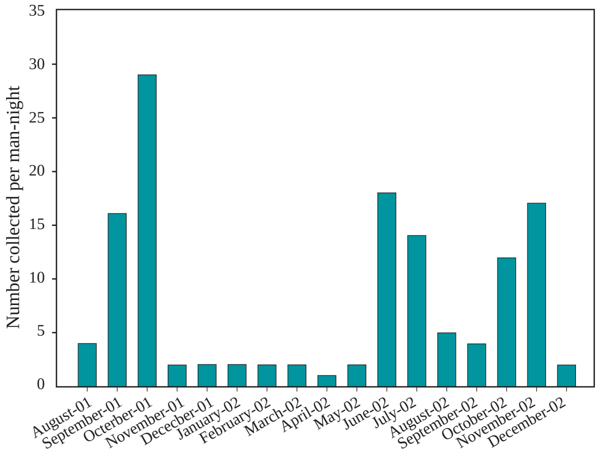

Fig 4–Seasonal comparison of the numbers of adult *Anopheles* collected from three villages in Tak Province from August 2001 to December 2002. Peak mosquito abundance (June-October) closely follows monthly rainfall (May-September) and trends in relative humidity.

Larval collections were made in and around the three villages to determine the types and abundance of habitats where anophelines occur. Collections consisted of systematically dipping with plastic dippers. The coordinates for each adult collection site and all larval habitats were recorded using a Global Positioning System (GPS) GeoExplorer III unit and processed with Pathfinder Office software (Trimble Navigation, 2001). Mosquito larvae were reared to adults, and identified to species.

The satellite data including LANDSAT Thematic Mapper (dated 22 January 1999) with a spatial resolution of 30 x 30 m and IKONOS images (dated 12 November 2001) with a spatial resolution of 1 x 1m images of the three villages and surrounding area have been acquired and used in a Geographical Information System (GIS) employing ERDAS software (ERDAS, 2002). Thematic maps were generated using ArcGIS 8.2

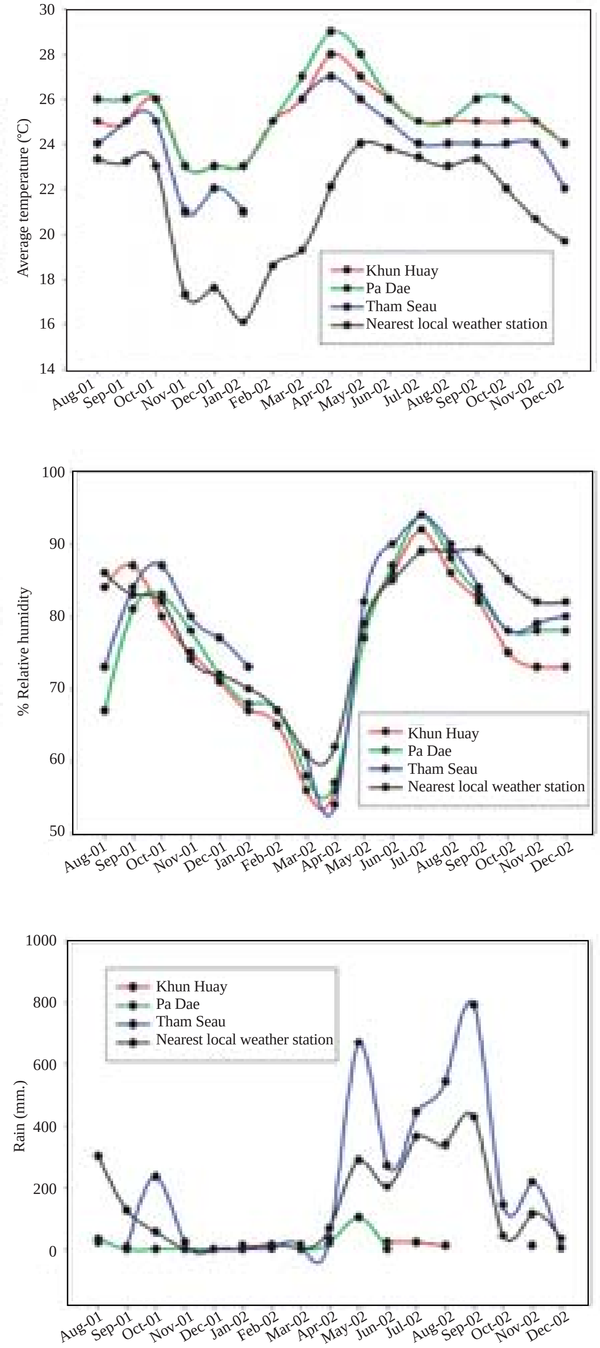

Fig 5–Monthly average temperature, relative humidity, and rainfall during August 2001-December 2002 among the three villages recorded by Stowaway Data Loggers and data from nearest local weather station.

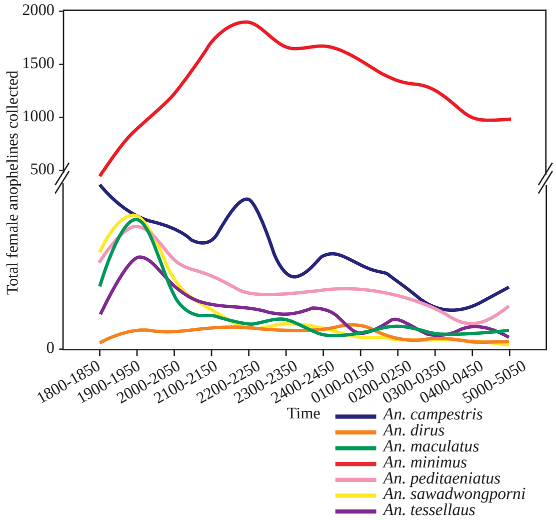

Fig 6–Nocturnal biting behavior of *Anopheles* females captured on humans at three villages in Tak Province from August 2001 to December 2002. *An. minimus* (red) was the most predominant species followed by *An. campestris* (blue).

(ESRI, 2002) software. A remote sensing (RS)-based GIS included spatial and temporal data in the form of geographic coverage and descriptive information in the form of relational databases associated with the mapped features.

Base topographical maps of the study site were also digitized and overlaid with data on the location of villages, houses and small streams in a GIS database using ArcGIS 8.2 (ESRI, 2002) software. Other features such as 20 m topographical contours and streamlines were also incorporated into the GIS. All digital data in the GIS are displayed in the UTM Coordinate system and georeferenced using the Indian 1975 Datum and Everest 1830 (1975 Definition) Ellipsoid Provincial border boundary. Thematic maps showing the proportion of adult and larval mosquito species at each of the 3 villages were created in the GIS using MapInfo (Mapinfo, 2000) and ArcGIS 8.2 (ESRI, 2002) software.

RESULTS

Distribution of malaria incidence per 100,000 populations for the year 2001 in different districts within 76 provinces of Thailand over

Table 2
Plasmodium-infected *Anopheles* mosquitos collected in the three villages between August 2001-December 2002.

Mosquito species	No. in pool	Date collected	Location	Hour	Species of *Plasmodium*	ELISA results	
						Abdomen	Head/ Thorax
An. minimus	1	17-Sep-01	Ban Pa Dae	2100-2200	*P. vivax*	-ve	+ve
An. minimus	4	27-Oct-01	Ban Tham Seau	2200-2300	*P. falciparum*	-ve	+ve
An. minimus	3	28-Oct-01	Ban Khun Huay	2300-2400	*P. vivax*	-ve	+ve
An. minimus	1	7-May-02	Ban Pa Dae	2400-0100	*P. vivax*	+ve	+ve
An. minimus	1	5-Jun-02	Ban Pa Dae	0100-0200	*P. vivax*	+ve	-ve
An. minimus	1	5-Jun-02	Ban Pa Dae	0100-0200	*P. vivax*	+ve	+ve
An. minimus	1	21-Aug-02	Ban Khun Huay	2300-2400	*P. vivax*	+ve	+ve
An. minimus	1	13-Nov-02	Ban Tham Seau	1900-2000	*P. vivax*	-ve	+ve
An. minimus	1	13-Nov-02	Ban Tham Seau	2200-2300	*P. vivax*	-ve	+ve

Table 3
The vectorial capacity and the Entomologic Inoculation Rate (EIR) comparisons between the wet season (August-October 2001 and May- October 2002) and dry season (November 2001-April 2002 and November-December 2002) for *An. minimus.*

	Wet	Dry
Human biting rate	52.10	18.55
Proportion parous	0.61	0.68
Daily survival	0.85	0.88
Vectorial capacity	14.42	10.42
Entomological Inoculation Rate (EIR)		
P. falciparum	0.005	0.000
P. vivax	0.023	0.010
Combined	0.028	0.010

a composite National Oceanographic and Atmospheric Administration satellite Normalized Difference Vegetation Index (NOAA/NDVI) data image is displayed in Fig 1. This image has been given false colors to show areas with high green-leaf biomass as a dark green color. In general when moving from green to light green to yellow to brown colors indicate decreasing vegetation density. Brown indicates no vegetation. Water shown in dark black color.

A total of 18,288 anopheline females were captured at the 3 villages during 68 nights of collecting (Table 1). The collections contained 21 species of *Anopheles*: *An. (Cel.) minimus* representing 86% of the collection, *An. (Ano.) campestris* Reid 4%, *An. (Ano.) peditaeniatus* Leicester 3%, *An. (Cel.) tessellatus* Theobald 2%, *An. (Cel.) maculatus* 2%, *An. (Cel.) sawadwongporni* Rattanarithikul and Green 2%, *An. (Cel.) dirus* 1%, and a combination of *An. (Cel.) kochi* Doenitz, *An. (Cel.) aconitus* Doenitz, *An. (Cel.) annularis* Van der Wulp, *An. (Ano.) argyropus* Swellengrebel, *An. (Ano.) barbirostris* Van der Wulp, *An. (Cel.) culicifacies*, *An. (Cel.) dravidicus* Christophers, *An. (Cel.) jamesii* Theoblad, *An. (Cel.) nivipes* Theobald, *An. (Cel.) philippinensis* Ludlow, *An. (Cel.) pseudojamesi* Strickland and Choudhury, *An. (Cel.) splendidus* Koidzumi, *An. (Cel.) vagus* Doenitz, and *An. (Cel.) varuna* Iyengar totaled 2%. The greatest number of adults was collected at Ban Khun Huay, followed by Ban Tham Seau and Ban Pa Dae. The proportion of *An. minimus* adults was greater

Table 4
Parity rates for of anophelines captured during human bait collections in Ban Khun Huay (August 2001- December 2002).

Species	% Gravid (# gravid)	% Nulliparous (# nulliparous)	% Parous (# parous)	% Parous & gravid (# parous & gravid)	Total determined
An. aconitus	7.1 (1)	28.6 (4)	64.3 (9)	71.4 (10)	14
An. annularis		61.9 (13)	38.1 (8)	36.4 (8)	21
An. barbirostris		42.9 (3)	57.1 (4)	57.1 (4)	7
An. campestris		61.5 (8)	38.5 (5)	38.5 (5)	13
An. dirus		60.0 (3)	40.0 (2)	40.0 (2)	5
An. jamesii		75.0 (3)	25.0 (1)	25.0 (1)	4
An. kochi	3.4 (1)	31.0 (9)	65.5 (19)	69.0 (20)	29
An. maculatus	8.3 (4)	52.1 (25)	39.6 (19)	46.9 (23)	48
An. minimus	4.4 (356)	38.2 (3,090)	38.2 (4,644)	58.5 (5,000)	8,090
An. nivipes	12.5 (1)	62.5 (5)	25.0 (2)	37.5 (3)	8
An. peditaeniatus	8.7 (13)	38.3 (57)	53.0 (79)	56.1 (92)	149
An. philippinensis		55.6 (5)	44.4 (4)	40.0 (4)	9
An. pseudojamesi		100.0 (3)			3
An. sawadwongporni	6.8 (6)	51.1 (45)	42.0 (37)	48.3 (43)	88
An. splendidus		27.3 (3)	72.7 (8)	72.7 (8)	11
An. tessellatus	8.3 (1)	50.0 (6)	41.7 (5)	50.0 (6)	12
An. vagus	20.0 (1)	20.0 (1)	60.0 (3)	80.0 (4)	5
An. varuna	8.0 (2)	32.0 (8)	60.0 (15)	65.4 (17)	25

Table 5
Parity rates for of anophelines captured during human bait collections in Ban Pa Dae (August 2001- December 2002).

Species	% Gravid (# gravid)	% Nulliparous (# nulliparous)	% Parous (# parous)	% Parous & gravid (# parous & gravid)	Total determined
An. aconitus		16.7 (1)	83.3 (5)	83.3 (5)	6
An. annularis		100.0 (1)			1
An. argyropus		75.0 (3)	25.0 (1)	25.0 (1)	4
An. barbirostris	16.7 (1)		83.3 (5)	60.0 (6)	6
An. campestris	6.4 (43)	40.2 (268)	53.4 (356)	56.4 (399)	667
An. dirus	20.6 (7)	26.5 (9)	52.9 (18)	69.4 (25)	34
An. jamesii			100.0 (1)	100.0 (1)	1
An. kochi		33.3 (7)	66.7 (14)	51.9 (14)	21
An. maculatus	12.5 (1)	50.0 (4)	37.5 (3)	50.0 (4)	8
An. minimus	6.3 (289)	38.0 (1,747)	55.7 (2,561)	58.5 (2,850)	4,597
An. nivipes		100.0 (2)			2
An. peditaeniatus	9.6 (28)	46.4 (135)	44.0 (128)	53.2 (156)	291
An. philippinensis			100.0 (2)	100.0 (2)	2
An. sawadwongporni	10.3 (4)	53.8 (21)	35.9 (14)	46.2 (18)	39
An. tessellaus	10.2 (25)	37.8 (93)	52.0 (128)	53.3 (153)	246
An. vagus	12.5 (1)	37.5 (3)	50.0 (4)	62.5 (5)	8
An. varuna		44.4 (4)	55.6 (5)	55.6 (5)	9

Table 6
Parity rates for of anophelines captured during human bait collections in Ban Tham Seau (August 2001- December 2002).

Species	% Gravid (# gravid)	% Nulliparous (# nulliparous)	% Parous (# parous)	% Parous & gravid (# parous & gravid)	Total determined
An. aconitus		66.7 (2)	33.3 (1)	33.3 (1)	3
An. annularis		50.0 (2)	50.0 (2)	50.0 (2)	4
An. barbirostris	3.8 (1)	38.5 (10)	57.7 (15)	57.1(16)	26
An. campestris	11.1 (6)	35.2 (19)	53.7 (29)	64.8 (35)	54
An. culicifacies			100.0 (1)	100.0 (1)	1
An. dravidicus		100.0 (1)			1
An. dirus	12.7 (10)	32.9 (26)	54.4 (43)	63.9 (53)	79
An. jamesii	33.3 (1)	33.3 (1)	33.3 (1)	66.7 (2)	3
An. kochi	13.8 (4)	20.7 (6)	65.5 (19)	79.3 (23)	29
An. maculatus	6.0 (14)	45.3 (106)	48.7 (114)	52.7 (128)	234
An. minimus	4.9 (104)	34.3 (735)	60.8 (1,302)	62.7 (1,406)	2,141
An. nivipes		100.0 (1)			1
An. peditaeniatus	6.6 (4)	44.3 (27)	49.2 (30)	54.0 (34)	61
An. philippinensis		100.0 (2)			2
An. pseudojamesi		100.0 (1)			1
An. sawadwongporni	3.4 (6)	50.6 (89)	46.0 (81)	48.6 (87)	176
An. splendidus		33.3 (1)	66.7 (2)	66.7 (2)	3
An. tessellatus		100.0 (4)			4
An. vagus	33.3 (1)	33.3 (1)	33.3 (1)	66.7 (2)	3
An. varuna		85.7 (6)	14.3 (1)	14.29 (1)	7

in Ban Khun Huay than in either Ban Pa Dae or Ban Tham Seau (representing 95%, 77%, and 76% of the specimens respectively). The second most common species in Ban Khun Huay was *An. peditaeniatus* (2%) while the second most common adult collected in Ban Pa Dae was *An. campestris* (11%) and Ban Tham Seau was *An. maculatus* (8%).

Adult *Anopheles* specimens were most commonly collected from June through October, closely following periods of greatest rainfall (Figs 4-5). Patterns of biting activity for all *Anopheles* are shown in Fig 6. Adult female mosquitos were collected on human bait during multiple collecting periods between 18 00 and 06 00 hours. All species exhibited a general decline in host-seeking activity after midnight, rising slightly at sunrise. There were marked differences in the biting activity among different species from the three villages studied. Among the major vectors, *An. dirus* was collected consistently between 19 00 and 02 00 hours. *An. minimus* was collected consistently throughout the night with a discernible peak at 22 00-23 00 hours. *An. maculatus* was collected consistently throughout the night without a clearly discernible peak. *An. sawadwongporni* was collected most often between 18 00 hours and midnight. Among the predominant non-major vectors, *An. campestris* was collected consistently throughout the night with two discernible peaks at dusk and 23 00 hours. *An. peditaeniatus* was collected consistently throughout the night without a clearly discernible peak. *An. tessellatus* was collected most often between 18 00 and 04 00 hours.

More than 60% of all the specimens captured were parous based on the coiling pattern of ovarian tracheoles (Tables 4-6). Sporozoites were detected by ELISA in either abdomen or head/thorax portions of nine pools of *An. minimus* specimens and no other species (Table 2). Eight pools were positive for *P. vivax* VK210 and one pool was positive for *P. falciparum*. The rate of infec-

Table 7
Breeding habitats of *Anopheles* larvae collected at Ban Khun Huay, Ban Pa Dae, and Ban Tham Seau (August 2001-December 2002).

Species	Fish pond	Flooded pool	Ground pool	Rice paddy	Rock pool	Sand pool	Seepage spring	Stream margin	Stream pool	Swamp	Swamp-ground pool	Wheel track	Total
An. aconitus	0	0	0	0	0	0	0	1	1	1	0	0	3
An. aitkenii gp	0	0	0	0	3	0	0	0	0	0	0	0	3
An. annularis	0	1	0	6	0	0	0	0	0	0	0	0	7
An. barbirostris	9	0	11	7	0	0	0	2	20	52	0	1	102
An. barbumbrosus	0	0	5	1	0	0	0	3	1	0	0	2	12
An. campestris	10	0	2	15	0	0	0	4	11	13	0	0	55
An. culicifacies	0	0	0	1	0	0	0	0	0	1	0	0	2
An. dirus	1	0	160	10	79	0	0	4	14	2	0	25	295
An. donaldi	0	0	0	1	0	0	0	1	0	0	0	0	2
An. hodgkini	0	0	0	0	0	0	0	0	0	2	0	0	2
An. jamesii	10	0	5	2	0	0	0	8	2	33	0	0	60
An. kochi	0	15	89	121	0	0	0	5	1	27	0	294	552
An. maculatus	0	0	22	9	5	28	45	103	36	4	0	3	255
An. minimus	4	0	127	177	33	2	6	1,277	828	68	7	34	2,563
An. nivipes	1	0	0	0	0	0	0	0	0	0	0	0	1
An. peditaeniatus	24	0	2	119	0	0	0	1	4	24	0	1	175
An. philippinensis	0	0	2	0	0	0	0	2	0	2	0	0	6
An. pseudojamesii	0	0	0	0	0	0	0	1	0	1	0	0	2
An. sawadwongporni	1	0	5	27	0	4	0	91	12	21	0	2	163
An. splendidus	0	0	0	0	0	0	0	5	0	1	0	0	6
An. subpictus	0	0	0	0	0	0	0	1	0	0	0	0	1
An. tessellatus	1	0	0	2	0	0	0	0	0	0	0	0	3
An. vagus	0	1	0	48	0	0	0	7	0	1	0	4	60
An. varuna	5	0	2	2	2	0	0	22	18	0	1	4	57

tion by *P. vivax* in Ban Khun Huay was 0.16% in August 2002 and in Ban Pa Dae was 0.06%, 0.39%, and 0.09% in September 2001, May 2002, and June 2002 respectively. Ban Tham Seau showed a rate of infection by *P. falciparum* of 0.06% in October 2001 and by *P. vivax* of 0.09% in November 2002.

Two hundred and ninety-one larval collections were made in and around the three villages during the periods when the biting collections were made. A total of 8,774 *Anopheles* mosquito larvae were collected from 12 categories of breeding habitats around the three villages during 17 months of collection. The species collected and their breeding places where they were found are listed in Table 7.

DISCUSSION

Significantly fewer adults were collected during periods of no rainfall and the lowest number of adults was found in April, the time of lowest relative humidity. Species diversity was also lowest during no rainfall, low humidity months, and *An. minimus* was the only species found in April and May. The Stowaway Data Loggers (Onset Computer Corporation, Pocasset, MA) temperature data was relatively higher than that recorded by the nearest local station while the humidity are about the same. Although we experienced some technical difficulty in recording the rain quantity by the Stowaway in Ban Khun Huay and Ban Pa Dae during May-October 2002, we observed that Ban Tham Seau had lower average temperature, higher relative humidity, and higher rainfall than the other two villages. The weather data could account for the difference in abundance of adult female and larval anophelines collected among the three villages studied.

The results of the present study suggest that *An. minimus* is the principal vector species in this area during the dry season and during much of the rainy season when populations of *An. dirus* are low in our study area. To analyze seasonal differences in vectorial capacity of *An. minimus* in our study area, between the wet season (August-October 2001 and May- October 2002) and dry season (November 2001-April 2002 and November-December 2002), we calculated the vectorial capacity as described by Rattanarithikul *et al* (1996). The vectorial capacity was somewhat higher in the wet season than in the dry season (14.4 and 10.4 respectively). The seasonal comparison between the entomologic inoculation rate (EIR) which is the number of human-biting mosquitos collected per person per day with CS antigen in the head and thorax as detected by ELISA, was also calculated. The EIR of *An. minimus* for *P. vivax* for the wet and dry seasons was 0.023 and 0.010, respectively (Table 3). For *P. falciparum* the EIR was 0.005 in the wet season, no EIR was calculated in the dry season since there were no *P. falciparum* positive specimens. *An. minimus* was considered to be the primary vector of malaria in Thailand until Scanlon and Sandhinand (1965) recognized *An. dirus* as a major vector. *An. minimus* was first found in Thailand by Payung-Vejjasastra (1935) and the capacity of this species to carry sporozoites of human plasmodia is well documented.

Most larvae were collected at sites near Ban Khun Huay, followed by Ban Tham Seau, and by the fewest larvae collected at Ban Pa Dae. This is in contrast to the adult collections, where more adults were collected at Ban Khun Huay and Ban Pa Dae than Ban Tham Seau. Although most immature sites contained *An. minimus* larvae, these sites were most often found near Ban Khun Huay and least often found near Ban Tham Seau. The results of larval collection are discussed in detail in Sithiprasasna *et al* (2003).

ACKNOWLEDGEMENTS

The authors would like to thank the Malaria Division, Ministry of Public Health for providing number of malaria cases, and the Civil Registration Division, Ministry of Interior for providing population data to calculate malaria incidence 2001. We also thank Mr Somporn Chanaimongkol, Mr Somsak Tiangtrong, Ms Ampornphan Kengluecha, Mr Boonsong Jaichpor, Mr Prasan Kankaew, Ms Patcharee Khongtak, and Mrs Suda Ratanawong for their assistance in the field-work. Funding for this project was provided by the Department of Defense - Global Emerging Infections Surveillance and Response System Unit located at the US Army Medical Component, Armed Forces Research Institute of Medical Sciences (USAMC-AFRIMS).

REFERENCES

Baimai V. Population cytogenetics of the malaria vector *Anopheles leucosphyrus* group. *Southeast Asian J Trop Med Public Health* 1988; 19: 662-80.

Belkin JN. The mosquitos of the South Pacific (Diptera: Culicidae). Berkeley: University of California Press, 1962: 412, 608.

Detinova T. Age-grouping methods in diptera of medical importance. WHO Monogr. 1962: 47p.

Division of Epidemiology, Thailand Ministry of Public Health. Preliminary Annual Summary. Epidemiological Surveillance Report. 2001-2002.

ERDAS Software Version 8.5 of ERDAS IMAGINE Tour guide and field guide. Atlanta GA: ERDAS Inc. 2002.

ESRI. ArcGIS 8.2 of ArcMap ArcCatalog and Arc Toolbox user's guide. Redlands, CA. 2001-2002.

Harrison BA. Medical entomology studies. XIII. The Myzomyia series of *Anopheles (Cellia*) in Thailand, with emphasis on intra-interspecific variations (Diptera: Culicidae). *Contrib Am Entomol Inst* 1980; 17: 1-195.

Harrison BA, Scanlon JE. Medical entomology studies-II. The subgenus *Anopheles* in Thailand (Diptera: Culicidae). *Contrib Am Entomol Inst* 1975; 12: 1-307.

Kittayapong P, Edman JD, Harrison BA, Delorme DR. Female body size, parity and malaria infection of *Anopheles maculatus* in Peninsular Malaysia. *J Med Entomol* 1992; 29: 379-83.

Malaria Division. Annual report of Ministry of Public Health. Ministry of Public Health, Thai Government, Bangkok. 2000-2002.

Mapinfo, Version 6 of Mapinfo user's guide, 2000. Troy, New York: Mapinfo Corporation, 2000.

Payung-Vejjasastra L. Malaria survey at Tung Song, Nakhon Si Thammarat. *Public Health Bull* 1935; 11: 658-66.

Rattanarithikul R, Green CA. Formal recognition of the species of the *Anopheles maculatus* group (Diptera: Culicidae) occurring in Thailand, Including the descriptions of two new species and a preliminary key to females. *Mosq Syst* 1986; 18: 246-78.

Rattanarithikul R, Panthusiri P. Illustrated keys to the medically important mosquitos of Thailand. *Southeast Asian J Trop Med Public Health* 1994; 25 (suppl 1): 1-66.

Rattanarithikul R, Konishi E, Linthicum KJ. Detection of *Plasmodium vivax* and *Plasmodium falciparum* circumsporozoite antigen in anopheline mosquitos collected in southern Thailand. *Am J Trop Med Hyg* 1996; 54: 114-21.

Rosenberg R, Andre RG, Somchit L. Highly efficient dry season transmission of malaria in Thailand. *Trans R Soc Trop Med Hyg* 1990; 84: 22-8.

Singhasivanon P, Thimasarn K, Yimsamran S, *et al*. Malaria in tree crop plantations in south-eastern and western provinces of Thailand. *Southeast Asian J Trop Med Public Health* 1999; 30: 399-404.

Scanlon JE, Sandhinand J. The distribution and biology of *Anopheles balabacensis* in Thailand (Diptera: Culicidae). *J Med Entomol* 1965; 2: 61-9.

Trimble Navigation Ltd. PFINDER Software, Version 2.80 of PFINDER Software user's guide. Sunnyvale, CA, 2001.

Wirtz RA, Sattabongkot J, Hall T, Burkot TR, Rosenberg R. Development and evaluation of an enzyme-linked immunosorbent assay for *Plasmodium vivax* VK 247 sporozoites. *J Med Entomol* 1992; 29: 854-7.

Wirtz RA, Zavala F, Charoenvit C, *et al*. Comparative testing of monoclonal antibodies against *Plasmodium falciparum* sporozoites for ELISA development. *Bull WHO* 1987; 65: 39-45.