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SEASONAL DENSITY AND MALARIA VECTOR COMPETENCE OF *ANOPHELES MINIMUS* AND OTHER ANOPHELINES AT A SHALLOW VALLEY IN NORTH THAILAND

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Abstract: Biting density of anopheline mosquitoes was monitored in both the rainy and dry seasons at 6 outdoor stations in two villages of north Thailand by 3 human and 1 water buffalo bait. A total of 19 *Anopheles* was collected. The most predominant species were *An. vagus* and *An. annularis.* They were followed by *An. hyrcanus* group, *An. minimus, An. aconitus, An. nivipes* and *An. kochi.* The rest species were not abundant. The fauna of *Anopheles* changed seasonally. *An. minimus, An. aconitus* and *An. barbirostris* were abundant in the dry season, while *An. maculatus* sensu lato, *An. kochi, An. tesselatus, An. vagus, An. annularis* and *An. hyrcanus* group were the rainy season *Anopheles.* The 'crude feeding index' was relatively high in *An. maculatus* and *An. minimus.* They were followed by *An. tesselatus* and *An. splendidus,* while that of *An. vagus* and *An. kochi* was less than 1/10 of *An. minimus. An. minimus* was regarded as the most important vector. The biting density of *An. minimus* was higher at the peripheral area of villages with scarce dwellings and rich vegetation. At stations with dense dwellings and adjoining to an open field, the density of *An. minimus* was low, and the malaria risk was probably higher.

Key words: anopheline fauna, vector competence, shallow valley, north Thailand

INTRODUCTION

In Thailand a clear peak of malaria cases appears in dry season, especially in November and December. The prevalence of potential malaria vectors affects this seasonality (Ismail *et al.,* 1978; Harbach *et al.,* 1987; Rosenberg *et al.,* 1990; Gingrich *et al.,* 1990). Five *Anopheles* were incriminated as the malaria vector in Thailand (Ismail & Phinichpongse, 1980; Malikul, 1988). Among them, *Anopheles minimus* has been considered as the primary vector at the mountain foot, forested foothills and forest fringes because the species is abundant at these areas, and preferably breeds in slow running streams located there (Ismail *et al.,* 1978; Harbach *et al.,* 1987; Ratanatham *et al.,* 1988).

These areas also were one of the most delicate and unstable areas because man has actively touched the natural environment by deforestation, human settlement, agricultural activities, and so on, and drastic environmental changes have occurred in many places. These changes must affect the bionomics of anopheline mosquitoes as suggested by Ismail *et al.* (1978) and Rao (1984). Therefore, it is quite necessary to re-examine the bionomics of anophlines occupying these areas. We conducted an investigation of anopheline fauna both in dry and rainy seasons from 1988 to 1990 in two villages located in a shallow valley on the forest fringe, aiming to clarify the seasonal density, the local distribution of major potential vector species, and the risk of bite to human, all of which were important parameters in the

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malaria epidemiology. Results obtained from the investigation were reported in this paper. In discussion, we also evaluated the vector competence of these species from the ecological point of view, and suggested an epidemiological scope of malaria in the mountain foot area.

MATERIALS AND METHODS

Two villages studied were in a shallow valley and surrounded by forested mountains, ca. 450 m above the sea level, 98° 52′ E and 19° 11′ N, 50 km north from Chiangmai city. Several clumps of dwellings were scattered within 4 km along an unpaved road. Reported number of population was 1335 individuals during the study period. Taking into the number and body size, human, water buffaloes and cows were main blood sources for malaria vectors in the study villages although dogs, cats, poultry and several swine were also reared. There were two slow running streams, which were a main breeding place of *An. minimus*. The flat part of land was used as terraced rice fields or dwellings. The lower part of slopes behind the flat area was mainly occupied by rhychee orchards, while the upper part was covered with the secondary forest.

A weekly population census for adult and larval mosquitoes was made from December, 1988 to February, 1989 in the dry season, from June to November, 1989 in the rainy season, and May to June, 1990 in the intermediate season in these villages. Adult collection was made at 2 stations selected among 6 stations in one night by a set of human and animal bait collections. In the human bait collection, 3 collectors exposed their legs to mosquito bite, sat outdoors, and caught all mosquitoes landed to them by an aspirator with a 50 minute collection followed by a 10 minute break from 18:00 to 24:00. In the animal bait collection, one water buffalo was tethered in a net ($4 \times 4 \times 2$ m), which was similar to one described by Service (1993), and one collector caught mosquitoes landing in and out of the net using an aspirator at every 15 minutes from 18:00 to 24:00. The mutual distance between stations was 300-500 m.

The crude feeding index (Service, 1993) on human was calculated using the following 4 parameters; the registered number of human population in the study area [1335], the number of water buffaloes plus cows counted by questionnaire [165], and the average numbers of anopheline mosquitoes caught by a human bait and by a water buffalo bait per half night. The risk of bite to human also was estimated by multiplying the crude feeding index by overall adult density.

Larval collection was made in 2 slow running streams targeting *An. minimus*. Four blocks each covering 300 m of the streams were selected near the adult collection stations. Twenty samples each composed by 30 dippings were collected at each regular

Table 1 Environmental conditions of 6 stations where an anopheline mosquito collection was made

Station	General topography	Vegetation	No. of houses	No. of water buffaloes & cows	Distance from a stream
1	An end of village B Adjoining to a rice field Distribution of dwellings; moderately dense Sparse canopy	Rice field	73	52	50 m (stream B)
2	A pass between Station 1 and 3 Forest located behind plantations	Sugar cane plantation Secondary forest	0	0	500 m (stream B)
3	Distribution of dwellings; Sparse, in a forest A bottle neck of a valley	Secondary forest	20	16	1000 m (stream A)
4	Adjoining to a wide rice field Widest part of a shallow valley Distribution of dwellings; moderately dense Sparse canopy	Rice field	115	55	300 m (stream A)
5	Adjoining to a wide rice field A center of village A Distribution of dwellings; dense Sparse canopy	Rice field	121	33	250 m (stream A)
6	An end of village A Distribution of dwellings; Sparse, in orchards Adjoining to a rice field Canopy composed mainly by orchards	Rice field Rhychee orchard	8	9	200 m (stream A)

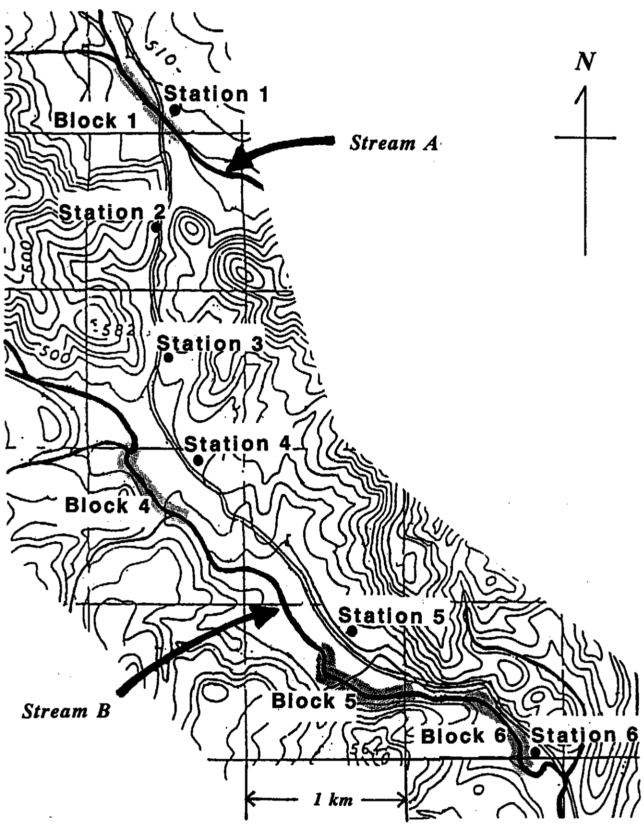

Figure 1 Map showing 6 stations for adult collection and 4 blocks for larval collection.

census. A round dipper sized 13.0 cm in diameter and 7.5 cm in depth was used.

Locations of 6 adult collection stations (1-6) and 4 larval collection blocks (1, 4-6) were illustrated in Fig. 1, and the environmental conditions of the stations were summarized in Table 1.

RESULTS

The anopheline fauna and the seasonal density of adults

During the study period, a total of 19 species of *Anopheles* was collected outdoors by human and water buffalo bait collections. They were *An. hyrcanus* group, *An. barbirostris* Van der Wulp in the subgenus *Anopheles*, and *An. minimus* Theobald, *An. aconitus* Donitz, *An. maculatus* sensu lato, *An. annularis* Van den Wulp, *An. nivipes* Theobald, *An. varuna* Iyengar, *An. kochi* Grassi, *An. tesselatud* Theobald, *An. vagus* Donitz, *An. splendidus* Koidzumi, *An. jamesii* Theobald, *An. stephensi* Liston, *An. philippinensis* Ludlow, *An. jeyporiensis* James, *An. pallidus* Theobald, *An. culicifacies* Giles and *An. dirus* Peyton & Harrison in the subgenus *Cellia.*

The most predominant species were *An. vagus* and *An. annularis.* They were followed by *An. hyrcanus* group, *An. minimus, An. aconitus, An. nivipes* and *An. kochi.* The rest species were not abundant. The average biting density (number of females per one bait per half night) of 12 dominant species, which were collected more than 200 females during the study period, were shown in Table 2.

In the dry season, *An. minimus, An. aconitus, An. annularis* and *An. hyrcanus* group were predominant, and the density of *An. minimus* caught by a human bait was outstanding. They were followed by *An. nivipes* and *An. barbirostris.* But the density of this second

Table 2 Average adult density (no./1 bait/half night) of 12 major anopheline mosquitoes collected by 3 human (H) and 1 water buffalo (B) baits in 2 mountain-foot villages in north Thailand

Season	No. of half nights	Bait	Average density												
			min	aco	niv	mac	koc	ann	var	tes	vag	hyr	bar	spl	Total
	53	B	48.9	54.8	29.4	3.3	8.5	48.1	3.6	2.3	7.4	43.6	27.7	3.5	281.1
Dry	47	H	3.1	1.8	0.4	0.2	0.1	0.7	0.1	0.1	0.0	0.5	0.3	0.1	7.4
		B+H	52.1	56.5	29.7	3.4	8.6	48.8	3.7	2.3	7.4	44.1	28.1	3.6	288.5
	53	B	8.3	2.7	26.6	15.4	40.7	133.8	0.0	12.5	138.6	73.2	5.7	6.4	464.8
Rainy	48	H	2.1	0.3	0.7	1.6	0.3	2.9	0.0	1.1	0.5	1.1	0.0	0.5	11.2
		B+H	10.3	3.1	27.3	17.0	41.0	136.7	0.0	13.6	139.1	74.4	5.7	6.9	476.0
	24	B	7.7	0.7	1.1	1.6	0.1	0.5	0.0	0.0	3.5	0.9	0.8	0.1	17.1
Intermediate	24	H	0.6	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9
		B+H	8.3	0.7	1.1	1.8	0.1	0.6	0.0	0.0	3.5	0.9	0.9	0.1	18.1
	130	B	24.7	23.6	23.0	7.9	20.1	74.3	1.5	6.0	60.2	47.8	13.8	4.1	307.3
Overall	119	H	2.2	0.8	0.4	0.8	0.2	1.4	0.0	0.5	0.2	0.7	0.1	0.3	7.6
		B+H	26.9	24.4	23.5	8.7	20.2	75.7	1.5	6.5	60.4	48.5	13.9	4.3	314.9

min, *An. minimus*; aco, *An. aconitus*; niv, *An. nivipes*; mac, *An. maculatus* sensu lato; koc, *An. kochi*; ann, *An. annularis*; var, *An. varuna*; tes, *An. tesselatus*; vag, *An. vagus*; hyr, *An. hyrcanus* group; bar, *An. barbirostris*; spl, *An. splendidus*.

abundant group was about a half of that of *An. minimus.* Other anophelines did not over 10 individuals per half night. The total anopheline density by human and water baffalo baits was 288.5 individuals.

In the rainy season, predominant species changed. *An. vagus* was strikingly abundant, though it was hardly collected by a human bait. *An. annularis* also was collected abundantly by a water buffalo bait. *An. maculatus, An. kochi, An. tesselatus* and *An. hyrcanus* group also were more abundant than in the dry season. On the other hand, the biting densities of *An. minimus* and *An. aconitus* in this season were evidently lower than those in the dry season. However, the density of *An. minimus* in human bait collection was the second highest. Total biting density by human and water baffalo baits (476.0) was much higher in the rainy season than that in the dry season.

In the intermediate season, May and June, all species were not abundant, and the anopheline mosquito fauna was poor. *An. minimus* and *An. maculatus* were the only 2 species collected by a human bait.

The difference of the adult density among stations

The frequency distributions of each species among stations were calculated using the average densities (Fig. 2). The predominant species could be classified into 4 groups depending on the frequency distribution. In the first group including *An. kochi, An. maculatus* and possibly *An. hyrcanus* group, a large proportion of samples were obtained at station No. 2 (Fig. 2a), while in the second group of *An. minimus, An. aconitus, An. varuna* and possibly *An. splendidus,* the distribution was characterized by high frequency at station No. 3 and 6, and by low frequency at No. 4 (Fig. 2b). In the third group including *An. annularis, An. nivipes* and *An. vagus,* the frequency at station No. 4 was the highest, and those at No. 2, 3 and 6 were low (Fig. 2c). *An. tesselatus* were clasified as the fourth group which was characterized by the high frequency at station No. 1 and 6, and low at station 4 and 5 (Fig. 2d).

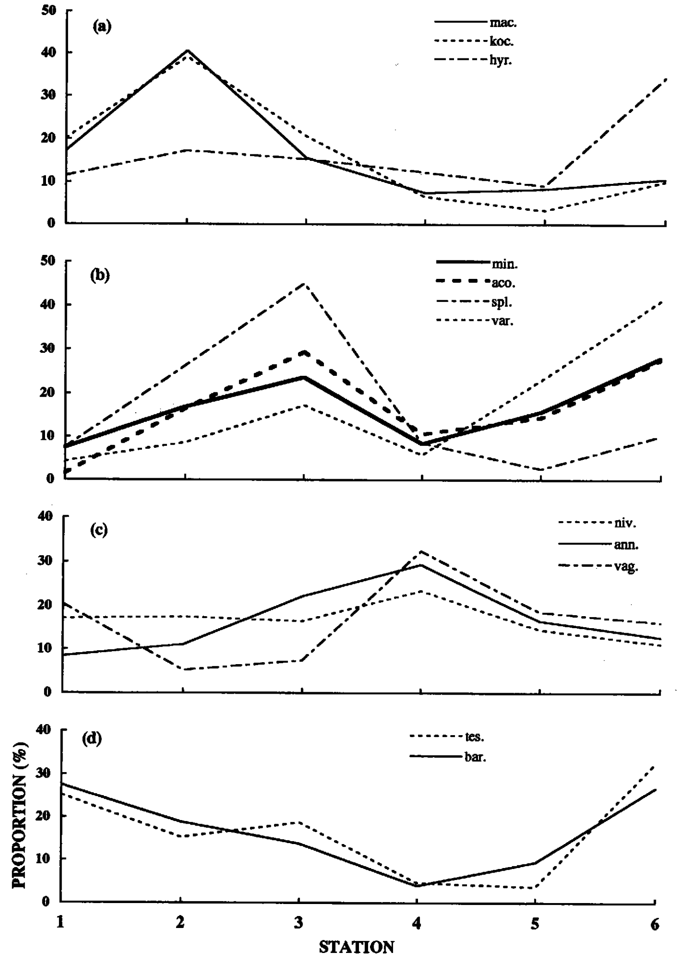

Figure 2 Proportion (%) of major anophelines (adult) at 6 stations.

Table 3 Average larval density (no./30 dips) of anophelines at 4 blocks of 2 slow running streams and average adult density (no./1 bait/half night) of *An. minimus* at the nearest stations to the blocks

Season	Parameter	Block and station						
		1	2	3	4	5	6	Average
Dry	Larval density	3.3	—	—	4.4	4.5	3.1	3.8
	% of *An. minimus* larvae	90.3	—	—	72.9	96.5	94.4	86.7
	% of young larvae	39.5	—	—	39.0	42.9	45.8	41.6
	Adult density of *An. minimus*	15.4	52.1	81.1	12.8	58.9	92.2	52.1
Rainy	Larval density	0.9	—	—	1.0	0.8	0.3	0.7
	% of *An. minimus* larvae	86.7	—	—	73.4	60.8	38.5	67.5
	% of young larvae	59.6	—	—	56.8	50.7	70.0	58.8
	Adult density of *An. minimus*	9.5	7.9	10.3	12.7	6.3	11.9	10.3

The larval population census in slow running streams

Results of the larval collection were summarized in Table 3. In the two slow running streams examined, the predominant species was always *An. minimus.* The proportion of this species among all anophelines collected in the streams was more than 90% in the dry season except at block 4. It decreased in the rainy season especially at blocks 5 and 6 but more than 2/3 of samples was still occupied by *An. minimus* on the average. Other collected species were *An. aconitus* and *An. hyrcanus* group. The former was more in the dry season, while the latter was more in the rainy season. The proportion of young larvae was higher in the rainy season. The average larval density of *An. minimus* among 4 blocks (only 3rd and 4th instar larvae were taken into account) was nearly similar to each other ranging only 3.1-4.5 per 30 dips in the dry season. It was reduced to less than 1.0 in the rainy season, and its reduction rate was larger from block 1 toward block 6. The frequency distribution of larval population of the species among blocks did not coincided to that of adult population among stations.

The host preference and the risk of bites to human

The human, baffalo and cow were considered as the main blood sources in the study villages. The host preference of females was roughly evaluated by the crude feeding index (Service, 1993) on human calculated by 4 parameters described in MATERIALS AND METHODS, and was shown in Table 4. Because the indices were rather small, all species encountered in this study were found to be zoophilic. Among 12 species, *An. minimus* and *An. maculatus* showed relatively strong preference to human. *An. tesselatus* and *An. splendidus* followed the former 2 species. *An. kochi* and *An. vagus* were strongly zoophilic because the indices of these species were less than 0.1 times of those in *An. minimus* and *An. maculatus.*

Multiplying the crude feeding index by the overall adult density shown in Table 2 enabled to estimate more realistic risk of bite to human by a certain malaria vector. The values calculated for each species were also shown in Table 4. *An. minimus* showed the largest value. This species was the most important malaria vector in the study area. The value of *An. annularis* was approximately 60% of *An. minimus.* Because of great abundance, this species should be dangerous in the rainy season. Both *An. aconitus* and *An. maculatus* showed moderate values. The values of other species were apparently smaller than these species.

DISCUSSION

Anopheles fauna in the study villages was mainly composed of 19 species, and the species diversity and density were higher in the rainy season. Five *Anopheles* were incriminated as the malaria vector in Thailand (Ismail & Phinichpongse, 1980; Malikul, 1988). Among these 5 species, 4 species except *An. sundaicus* were collected in our study. *An. dirus* is the primary vector in deep forests and mountains with serious malaria endemicity (Ismail *et al.,* 1974, 1975; Gingrich *et al.,* 1990; Rosenberg *et al.,* 1990). Although this species is highly anthropophilic, only one adult female was caught by human bait in our study area located in a shallow valley of forest fringes and surrounded by low mountains. The scarcity of *An. dirus* in forest fringes was also confirmed by Ismail *et al.* (1978). Therefore, the important malaria vectors in this area should be the rest of 3 species, *An. minimus, An. aconitus* and *An. maculatus* sensu lato.

The calculation of the crude feeding index and the risk of bite to human suggested that *An. minimus* was the most important malaria vector in the study area because of both its relatively high density and high feeding index to the human bait. The highest crude feeding index in *An. maculatus* was noteworthy. Although the crude feeding index was not large, *An.*

Table 4 Crude feeding index on human* and the risk of bite to human† in major anopheline mosquitoes

Parameter	min	aco	niv	mac	koc	ann	var	tes	vag	hyr	bar	spl
Crude f. ix.	0.0110	0.0043	0.0023	0.0119	0.0010	0.0024	0.0040	0.0094	0.0004	0.0017	0.0013	0.0078
Ratio to *min*	1.0000	0.3953	0.2105	1.0786	0.0925	0.2186	0.3623	0.8570	0.0398	0.1587	0.1211	0.7116
Risk of bite	0.2963	0.1061	0.0543	0.1028	0.0206	0.1821	0.0061	0.0611	0.0264	0.0846	0.0185	0.0339
Ratio to *min*	1.0000	0.3580	0.1833	0.3470	0.0695	0.6146	0.0205	0.2062	0.0891	0.2856	0.0626	0.1143

min, *An. minimus*; aco, *An. aconitus*; niv, *An. nivipes*; mac, *An. maculatus* sensu lato; koc, *An. kochi*; ann, *An. annularis*; var, *An. varuna*; tes, *An. tesselatus*; vag, *An. vagus*; hyr, *An. hyrcanus* group; bar, *An. barbirostris*; spl, *An. splendidus.*

*Density in human bait × water buffalo plus cow populations/density in buffalo bait × human population

†Crude feeding index × overall density

annularis was probably more important than *An. aconitus* in the study area because the species was rather abundant throughout the year. It should be noted that *An. annularis* was regarded as an important malaria vector in some localities in Sri Lanka (Ramasamy *et al.*, 1992) and India (Rao, 1984).

Four types of frequency distribution of *Anopheles* were observed in the study area. *An. minimus* was abundant at a peripheral part of villages with scarce dwellings and rich vegetation. In contrast with this, this species was not abundant at a part with dense dwellings. These facts suggested that malaria risk transmitted by *An. minimus* might be larger in scarcely populated area such as periphery of villages and between villages. It was possibly due to more attacks by the species to limited blood sources, and concentrated distribution to the abundant resting places.

The topographic and vegetational characters of the study area, such as locating in a shallow valley with slow running streams and adjoining a forest fringe, are widely observed in inland of southeast Asia. Drastic environmental changes also have been occurred in this part of Asia by deforestation, human settlement, agricultural activities, and so on (e.g. Walsh *et al.*, 1993). These changes must affects the bionomics of anopheline mosquitoes, especially *An. minimus* as suggested by Ismail *et al.* (1978). A continuous monitoring of both the environment and the vectors by a standardized method is still necessary to yield more harmonious vector control strategy.

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