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# Studies on Malaria and Responses of *Anopheles balabacensis balabacensis* and *Anopheles minimus* to DDT Residual Spraying in Thailand.[1] Part I. Pre-Spraying Observations.

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*Abstract*

The malaria eradication programme in Thailand has been facing difficulties in interrupting transmission in forested and foothill areas where the malaria vectors, *A. b. balabacensis* and *A. minimus* are prevalent. As a result, field studies on the responses of the two malaria vectors to the attack measures with DDT residual spraying were undertaken in a typical forest fringe and foothill area in northern Thailand. The studies also included observations on other epidemiological factors. A field station, similar to one of the village hamlets, was established in the selected site. The studies were planned on two periods, one before and the other after the application of DDT residual spraying. Results obtained during the first period, for 21 months, are presented in this paper. The present studies revealed important findings on both vectors in respect to: seasonal prevalence, indoor and outdoor contact with man, breeding places, biting cycle, age composition and sporozoite infection. Efforts to locate the day-time resting places were also made. The house frequenting behaviour of both vectors was studied by the use of two different types of human-baited experimental huts. *A. minimus* showed stronger exophagic and exophilic tendencies than *A. b. balabacensis,* but nevertheless the latter proved to be the more efficient vector. Results with *A. minimus* are contrary to the earlier findings on this vector in the plains. Mass blood surveys were carried out at monthly intervals. Blood films were also collected at the field station which thus functioned as a passive case detection post as well. All malaria cases were investigated thoroughly. Results of the last observations have thrown light on the endemicity of malaria, transmission season, prevalent malaria parasite species and localities where infection took place.

## 1. Introduction

In forested and foothill areas of Thailand where *A. b. balabacensis* and *A. minimus* are the main vectors, the interruption of malaria transmission has been facing difficulties. The first vector is exclusively a forest mosquito, the second breeds primarily in water streams at foothills. Previously *A. minimus* was believed to be the only main malaria vector in Thailand. Detailed studies of this species

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were carried out in 1949/50 by Sambasivan, Bhatia, Pranich and Notananda (unpublished report to WHO, 1953)[5] who reported that in the flat project area with rice cultivation, *A. minimus* was an endophilic species, biting man mostly late at night. It was highly susceptible to DDT residual spraying. In the following years of the malaria campaign *A. minimus* responded favourably to the attack measures, wherever conditions resembled those of the project area. However, signs of variations in the behaviour of *A. minimus* were noted in foothill areas especially in the forested ones. Of the role of *A. b. balabacensis* in malaria transmission little was known in the first years of the malaria campaign, when the movement of the human population in the forest was rather limited. With the increase and expansion of the rural population and their increasing penetration of the forest, the importance of *A. b. balabacensis* became apparent. This situation required an investigation of this species' behaviour and response to the attack measures especially where interruption of transmission could not be achieved. SCANLON & SANDHINAND (1965) studied the biting and resting habits of *A. b. balabacensis* in an area in Bang Lamung District of Cholburi Province and came to the conclusion that malaria would be extremely difficult to control or eradicate in the areas where this species was the vector. Similar conclusions were also reached by EYLES et al. (1964) from their studies in neighbouring Cambodia (Khmer).

From the point of view of the malaria programme in Thailand, these results were not conclusive and the need was felt for extensive operational studies on the vector and on important epidemiological factors. For this purpose the present studies were initiated in a typical foothill area in the forest fringe where both *A. b. balabacensis* and *A. minimus* were prevalent. The studies were to be conducted over two major periods, one before and the other after application of DDT residual spraying. Results obtained during the first period, from July 1970 to March 1972, are presented in this paper.

## 2. The study area, background information on malaria

Based on the result of field surveys, a site was chosen near village 7, Wang Nuken Canton, Wang Thon District, Pitsanuloke Province, northern Thailand. This site is situated in the forest fringe, some 250 meters from the nearest houses of village 7. This locality consists of approximately 180 houses and huts, scattered in the form of small hamlets. The part of the village neighbouring the selected site consists of 51 houses and huts with a population of about 250, situated in the narrow valley of Lam Kradon. The valley is flanked on the east and on the west by two ranges of hills. In the north it ends at the Mae Nam Khek river close to which runs the highway of Pitsanuloke–Lomsak. The southern, upper part of the valley is forested (Fig. 1). Originally the whole valley and the surrounding hills were covered by dense forest but since a few years ago, especially after the construction of the highway, clearance of the forest has advanced gradually. At present the cleared part of the valley is about 4 km long and its southern end is separated from the forested area by a permanent small water stream which runs from south to north, near the foothills on the eastern side of the valley, until it flows into Mae Nam Khek river.

Conditions in the study area are ideal for the breeding of *A. b. balabacensis* and *A. minimus*. The presence of these two important malaria vectors was responsible for a significant degree of transmission. During 1968 and 1969 the

[5] Report of Thai Government/WHO/UNICEF Malaria Control Demonstration Project Thailand, SEA/MAL 1 (unpublished report to WHO).

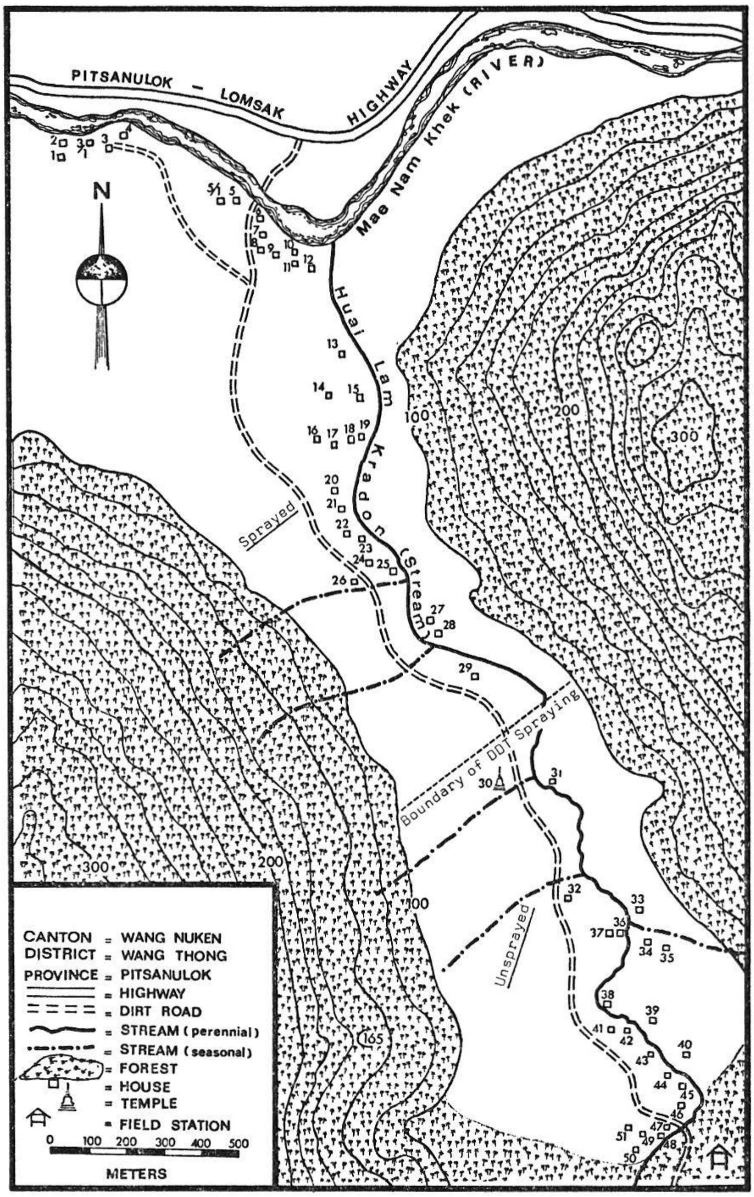


*Fig. 1.* Entomological study area in Lam Kradon locality of village 7, during 1971.

annual parasite incidence (API) of the whole canton, consisting of seven villages, was 47.2 and 32.9 respectively, whereas in village 7 alone it was 70 and 37.7 respectively. The activities of the villagers also facilitated malaria transmission in the area. Besides farming, their main occupation, people move into the neighbouring forest for cutting wood, hunting, clearing spots inside the forest for agriculture, collecting wild fruits and extracting plant oil from wild trees. Seasonally or occasionally they sleep inside temporary huts. Such activities in the forest made it difficult for the malaria personnel to trace the villagers, to find and treat the patients, and to detect temporary shelters and spray them in time.

Residual spraying with DDT in the area started in 1961. From 1961 to 1964 the area received one cycle of DDT spraying per year with a dosage of 2 g/m$^2$. Since 1965, two cycles per year were applied using the same dosage. For the purpose of this study, and in order to permit the density of the vectors to build up, spraying was interrupted within a radius of 2 km from the field station since the beginning of 1970.

## 3. Methods

### 3.1 The field station

It was decided to create a hamlet simulating local conditions which we refer to here as the field station. This station, as shown in Fig. 2, consists of:

i. A well constructed large house (HQ) serving as headquarters, comprising laboratory, verandah, store room, kitchen and sleeping quarters for professional staff. The house is raised one metre above the ground and built of the material in common local use. It measures about 50 square meters, rises to 3.5 metres in the centre and is made of a wooden floor, walls of split bamboo and a thatched roof.

ii. A hut (X) for night-time mosquito collections on human baits. It measures 2.40 by 2.40 metres, is 2.70 metres high in the centre and has a thatched roof. Floor and walls are made of split bamboo. Also it is raised one metre above the ground.

iii. Three huts (HI) and three tents serving as sleeping quarters for the rest of the staff. These huts resembled hut (X) but were different in size.

iv. A separate large kitchen (K) for the use of staff.

v. Six experimental huts (A, B, C, D, E, F) for studies on the house frequenting behaviour of mosquitoes.

The large house resembled houses in the village while the huts resembled those usually seen in the forest. In order to create conditions similar to the ones found in the village, two buffalos were hired and kept in an open shed (Ani) during night-time. Moreover 15 pit-shelters were made in and around the field station for the collection of day-time resting mosquitoes.

With the co-operation of the local population, forest clearance during the period of study was reduced to a minimum.

### 3.2 Entomological techniques

#### 3.2.1 Collection of mosquitoes on human baits

These were made by insect collectors capturing mosquitoes from themselves. From July 1970 to May 1971, indoor collections were carried out inside the laboratory of the large house and since then inside hut X. Outdoor collections were always done at fixed place. During periods of rainfall, insect collectors working outdoors moved under a plastic roof supported by four bamboo poles, and collections continued there. The use of plastic roof was adopted from the habits of local hunters who occasionally use similar shelters during their stay in the forest. Eight insect collectors worked from 18.00 to 06.00 hours under regular supervision. They worked in pairs, each pair for only half of the night. The insect collectors were rotated on the two sites and during both halves of the night. They worked for 50 minutes and had a rest of 10 minutes each hour. The captured mosquitoes were kept in test tubes in an ice box. In principle collections were carried out on seven consecutive nights every second week.

Mosquitoes collected on the human bait were identified and dissected for age grouping and sporozoite infection on the following morning. The latter technique was only started in June 1971. Age grouping was made by examining tracheoles of the ovary (DETINOVA, 1962). Gland dissections were carried out on parous females only for detection of sporozoites.

#### 3.2.2 Experimental huts

In order to study the house frequenting behaviour of the vector species under normal conditions and later their response to DDT spraying, two types of human

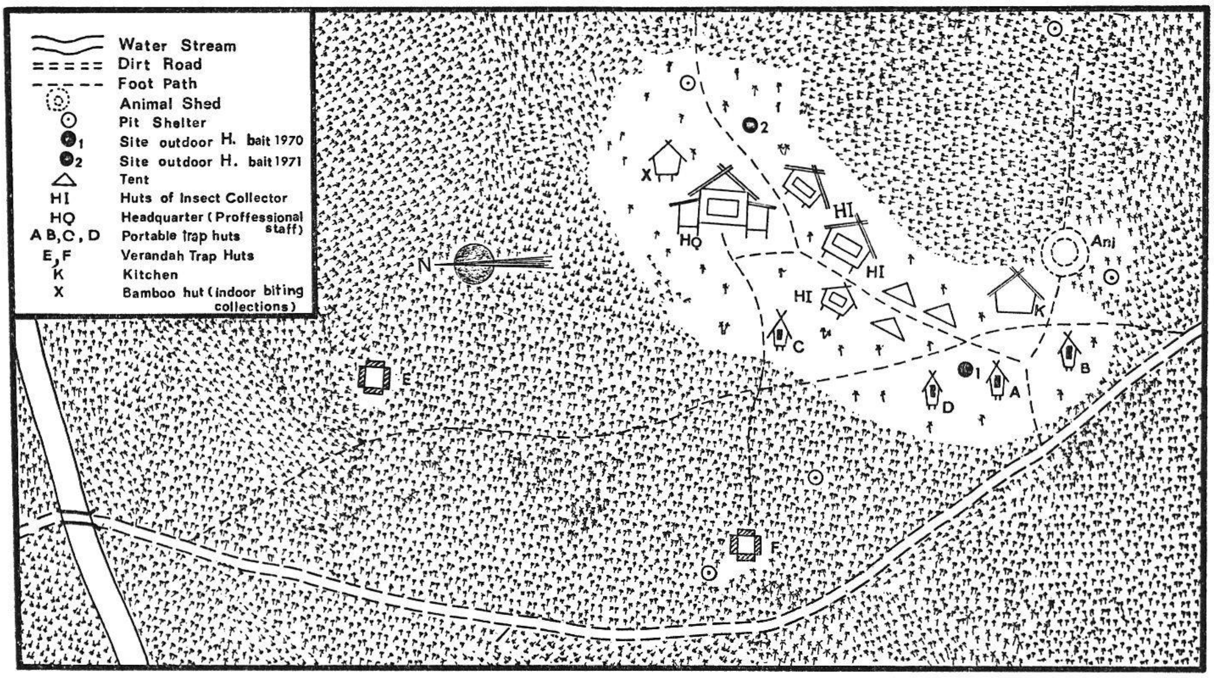


*Fig. 2.* Sketch map of entomological field station in the forest fringe of Lam Kradon Locality of village 7, Wang Thong, Pitsanulok, during 1971.

baited experimental huts were constructed: a verandah-trap hut and a portable-trap hut.

*i. Verandah-trap hut*

Two verandah-trap huts, similar to the one described by Smith (1965) in East Africa, but with slight modifications were constructed. The distance between both huts was approximately 70 meters. In the construction local building materials were used, i.e. bamboo mats for the walls and the internal lining of the roof, thatch for the roof and hardboard for the floor. Following the technique of Smith (1965), two opposite verandahs were put in operation and alternated with the other two verandahs on a weekly basis. Window traps, one on every side, were fitted in each of the operated verandahs. Three children and one adult served as baits in each hut. They entered the hut at 19.00 hours and stayed throughout the night. At 06.00 hours two insect collectors collected mosquitoes trapped in the verandahs and window traps, and recorded mosquitoes resting inside the hut. Mosquitoes were identified, recorded and classified as unfed, fed or gravid. The total number of mosquitoes leaving by the eaves was assumed to be twice the number caught in the two verandah traps. In order to avoid differences in the human attractiveness, baits rotated on a daily basis on both experimental huts. The two verandah-trap huts were marked E and F.

*ii. Portable-trap hut*

In 1970, three portable-trap huts with two window traps each were built as described by Chang (unpublished report to WHO, 1958) [6] in Sabah, East Malaysia. They were installed in the field station about 80 meters apart from each other.

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[6] WHO Assignment Report, Malaria Pilot Project in North Borneo, August 1955, unpublished report to WHO.

In 1971 one more hut was added and the huts were then brought closer to each other. Certain alterations were also introduced in order to simulate, as far as possible, the local conditions. The dimensions of the huts were 8 feet long, 4.5 feet wide and 7 feet high at the eave. The floors were raised one meter from the ground. The huts were constructed of the same materials as the verandah-trap huts. Window openings of about 1.5 feet square were located at the centre of the wall on the two short sides of the hut. A detachable window trap was installed over the window opening. The door was situated at one corner of the hut. The entrance for the mosquitoes was provided with two louvre boards, one on each side, forming a series of transversal wooden slats, set one above the other at an inch and a quarter apart, sloping 30° downwards and outwards, more or less like shutter windows with fixed slats. The overall dimensions of the louvre board were 6.5 feet high, 2.5 feet wide and 3 inches thick. The slats were painted black. Owing to the downward sloping of the black slats, very little light could enter through the louvre board. A board made of plywood was also installed 15 cm behind the louvre in order to decrease the entrance of light to the minimum. When observed from inside during the night time the window traps were the source of light. In each hut two children served as baits and rotated on the four huts. Every night they entered at 19.00 hours and left at about 06.00 hours. Collection of mosquitoes from window traps was carried out by two insect collectors either on hourly basis starting from 20.00 hours until 06.00 hours, or only once in the morning depending on the density of mosquitoes. The doors of the huts were kept closed throughout the night until 06.00 hours when an insect collector entered the huts searching for indoor resting mosquitoes. Early in the morning mosquitoes collected from the window traps or while resting indoors were identified and their abdominal condition was examined and recorded. The two window traps in each hut operated only as exit traps. Although they were detachable they were kept all the time attached to the windows. Along with the huts they were cleaned once a day at 17.00 hours from mosquitoes and other insects. The four portable-trap huts were marked A, B, C and D.

### 3.3 Epidemiological surveys

Mass blood surveys covering the population living in the field station, the neighbouring hamlet along the fringe of the forest, and the area extending to approximately 2 km north of the camp were carried out at monthly intervals. The population consisted of the station's employees, village residents and their visitors. Patients going to or coming from the forest had their blood examined at the field station, which thus functioned as a passive case detection post as well.

All positive cases were investigated thoroughly so as to elucidate origin and time of infection. For confirmation, visits were also made to the places where the infections had presumably occurred.

During the mass blood surveys, all fever cases were given presumptive treatment consisting of 600 mg chloroquine (base) and 50 mg pyrimethamine. Staff and hired human baits were given weekly prophylactic doses of 600 mg chloroquine (base) and 15 mg primaquine (base). Falciparum infections among the latter group were treated radically with 1,000 mg sulfadoxine, 50 mg pyrimethamine and 75 mg primaquine (base). Cases among the village population were treated according to the standard regimen of the malaria programme, i.e. 1,500 mg chloroquine (base) and 75 mg primaquine (base). If the infections failed to respond, the same regimen was applied as that used in field station personnel. All aforementioned doses are adult doses. Due adjustments were made for the younger age groups.

### 3.4 Meteorological data

Records on rainfall, atmospheric temperature and relative humidity were regularly taken in the field station. The amount of rainfall was read twice daily at 06.00 hours and 18.00 hours while temperature and relative humidity were recorded by means of a thermohygrograph.

## Results

### 4.1 Human bait collections

#### 4.1.1 Seasonal prevalence

Data on the total number of mosquitoes collected on the human baits indoors and outdoors for each month have been used for illustrating the seasonal prevalence of *A. b. balabacensis* and *A. minimus* during the period of study which extended over 21 months. The monthly indices are expressed as the average number of mosquitoes per man per night. The results obtained are given in Table (1) and presented in Fig. 3 along with the meteorological data.

The rainfall records show that the study area has a rainy season extending from May to September/October and a dry season from October/November to April. A marked correlation was noted between the rainfall pattern and the population density of *A. b. balabacensis*. During the rainy season *A. b. balabacensis* was prevalent at a high density, with a peak in June/July, while the density markedly decreased during the dry season until the species became very scarce due to the lack of breeding sites in the area. A survey of the breeding places revealed three types, two natural and one artificial:

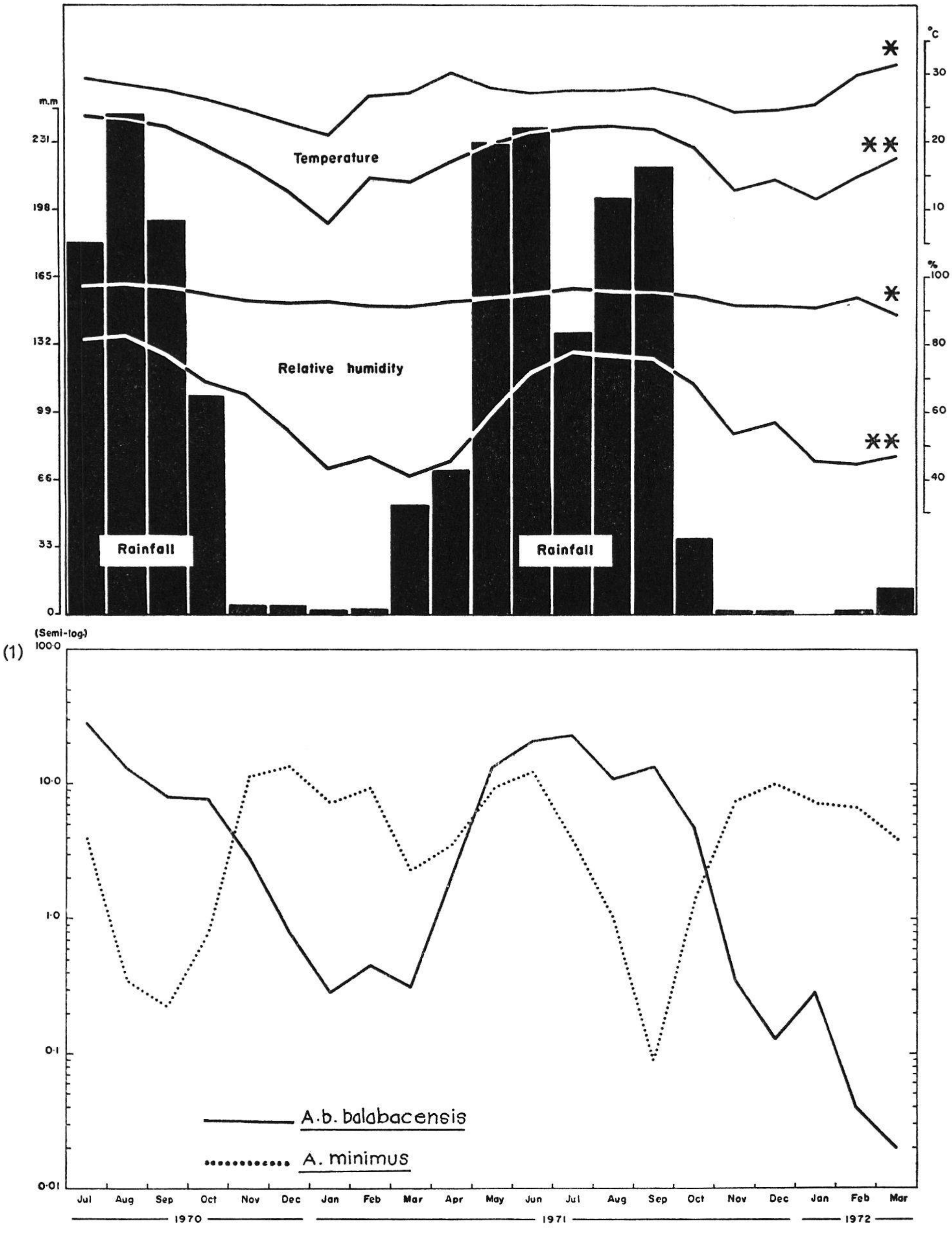
a. Shallow seepage pools at the sides of seasonal water courses, partially shaded, with rather clear water and decaying foliage at the bottom. Such streams were located inside the forest and also near the village houses.
b. Similar shallow pools as above but at the sides of the permanent stream near the field station. The location of these pools changed during the rainy season according to the water level in the stream.
c. Cement basins located under the verandah-trap huts for protection against ants, containing clear water with organic matters at the bottom and partially shaded.

Temporary isolated pools were formed during heavy rainfall but due to the porous texture of the soil they did not hold water for more than a few hours after rain. Therefore, they did not serve as breeding sites for the vector.

*Table 1. A. b. balabacensis* and *A. minimus* collected on human baits indoors and outdoors during the period July 1970 to March 1972

| Serial No. | Month and year | *A. b. balabacensis* | | | *A. minimus* | | |
|---|---|---|---|---|---|---|---|
| | | indoors average/ man/night | outdoors average/ man/night | total average/ man/night | indoors average/ man/night | outdoors average/ man/night | total average/ man/night |
| | 1970 | | | | | | |
| 1 | July | 44.14 | 11.82 | 27.98 | 4.46 | 3.21 | 3.84 |
| 2 | Aug. | 20.50 | 5.60 | 13.05 | 0.33 | 0.37 | 0.35 |
| 3 | Sep. | 9.97 | 6.19 | 8.08 | 0.28 | 0.16 | 0.22 |
| 4 | Oct. | 8.12 | 7.47 | 7.79 | 0.74 | 0.85 | 0.79 |
| 5 | Nov. | 1.47 | 4.37 | 2.92 | 4.73 | 17.73 | 11.23 |
| 6 | Dec. | 0.07 | 1.50 | 0.79 | 6.43 | 20.80 | 13.61 |
| | 1971 | | | | | | |
| 7 | Jan. | 0.07 | 0.50 | 0.29 | 5.64 | 9.00 | 7.32 |
| 8 | Feb. | 0.14 | 0.79 | 0.46 | 6.29 | 12.29 | 9.29 |
| 9 | Mar. | 0.29 | 0.36 | 0.32 | 1.60 | 3.00 | 2.29 |
| 10 | Apr. | 1.93 | 2.21 | 2.07 | 3.57 | 3.57 | 3.57 |
| 11 | May | 13.29 | 13.80 | 13.55 | 8.14 | 10.30 | 9.21 |
| 12 | June | 21.79 | 20.93 | 21.36 | 11.80 | 12.57 | 12.18 |
| 13 | July | 25.36 | 20.00 | 22.68 | 4.82 | 2.61 | 3.71 |
| 14 | Aug. | 12.11 | 10.32 | 11.21 | 1.00 | 0.96 | 0.98 |
| 15 | Sep. | 12.18 | 14.75 | 13.46 | 0.18 | 0.00 | 0.09 |
| 16 | Oct. | 3.96 | 5.75 | 4.86 | 1.50 | 1.43 | 1.46 |
| 17 | Nov. | 0.18 | 0.54 | 0.36 | 6.46 | 8.54 | 7.50 |
| 18 | Dec. | 0.11 | 0.14 | 0.13 | 6.75 | 12.61 | 9.68 |
| | 1972 | | | | | | |
| 19 | Jan. | 0.14 | 0.43 | 0.29 | 6.36 | 8.46 | 7.41 |
| 20 | Feb. | 0.0 | 0.07 | 0.04 | 4.50 | 8.93 | 6.71 |
| 21 | Mar. | 0.0 | 0.04 | 0.02 | 3.07 | 4.68 | 3.88 |

Studies on *A. minimus* have shown that the pattern of its seasonal prevalence differs from that of *A. b. balabacensis*. The species appeared in high density at the beginning of the dry season in the month of November and remained at high density from November to February, i.e. during the major part of the dry season. The density dropped in March/April, the later part of the dry season. A second peak, but of shorter duration, appeared in the early part of the rainy season in May/June, followed by a decline in July and a further decrease to reach the lowest density in August/September. The breeding habitat of *A. minimus* differs from that of *A. b. balabacensis*. Rainfall has lesser and indirect effect on the breeding and hence on the seasonal prevalence of *A. minimus*. The typical breeding sites of *A. minimus* are




(1) Average no. of bites per man/night
* mean maximum temperature and mean maximum relative humidity
** mean minimum temperature and mean minimum relative humidity

*Fig. 3.* Seasonal prevalence of *A. b. balabacensis* and *A. minimus,* and meteorological factors as recorded from the field station.

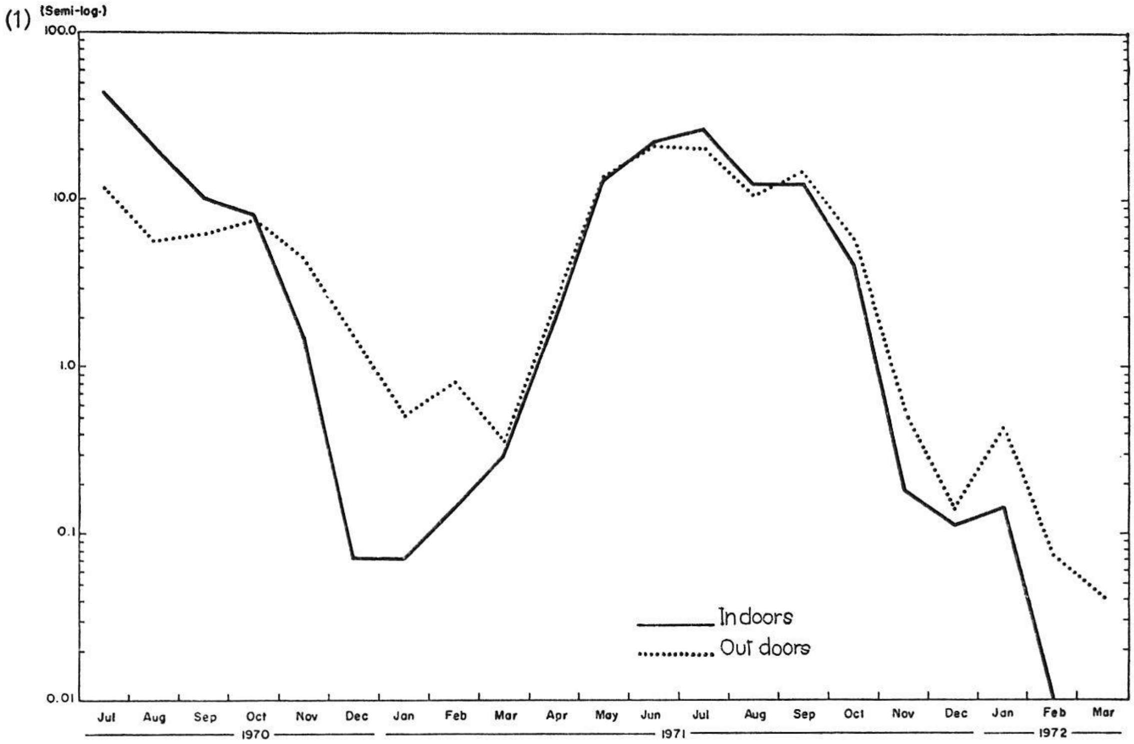
the grassy edges along clear running water streams, seepage and perennial rivers (THOMSON, 1940). It seems that the drop in the population density of the species during the second half of the rainy season is caused by the washing out of the breeding places by floods which usually occur at this time of the season, while the second drop observed during the later part of the dry season may be due to the partial drying up of the breeding places. This follows nearly the same pattern of the seasonal prevalence of *A. minimus* previously reported from south China and Cambodia (Khmer). In China a high peak was reported in October and November and a smaller one in May and June (CHOW, 1948), whereas in Cambodia (Khmer) one peak lasted from November to January and the other peak from May to July (CHOW, 1970).

Rather stable temperature and relative humidity during the rainy season coincide with the seasonal prevalence of *A. b. balabacensis* whereas a wider fluctuation of both meteorological factors coincides with the important peak of *A. minimus* during the dry season, i.e. November–February.

#### 4.1.2 Man-vector contact indoors and outdoors

The indoor and outdoor collections of *A. b. balabacensis* and *A. minimus* on human baits were carried out with the main objective of studying the endophagic and exophagic tendencies of the vector species throughout the season. In Table (1), details of the biting density per man per night are given for each indoor and outdoor site for each month. As mentioned earlier the site of indoor and outdoor collections was changed from the large house (HQ) to a small hut (X) since June 1971 and consequently the site of outdoor collections was also changed.

Results with *A. b. balabacensis* (Fig. 4) show that in 1970, when observations covered only a part of its season, the highest biting density was recorded in the month of July with 44.14 and 11.82 per man/night for indoor and outdoor sites, respectively. During the following four months the biting density steadily decreased and the decline was more marked indoors than outdoors. In comparing the indoor and outdoor biting densities, it was noted that during July–August the indoor density was about 4 times higher than that observed outdoors. In September the difference was not more than 1.5 times. In October the biting densities indoors and outdoors were nearly equal while during the following month there was a reversal as the biting density outdoors exceeded that of indoors by more than 3 times. During the off-season period from December 1970 to March 1971 the overall densities were very low, especially indoors. The first signs of increasing density were noted in April. The biting densities indoors and outdoors during that month, as well as in May and June, were nearly equal, while in July and




(1) Average no. of bites per man/night

*Fig. 4.* Man-vector contact in *A. b. balabacensis,* indoors and outdoors.

August the density indoors slightly exceeded the density outdoors, but not as much as recorded during the same period in the previous season. During September and October the results reversed and a small difference in favour of the outdoor collections was recorded. From November onwards the biting densities became very low and the results were similar to those of the previous season.

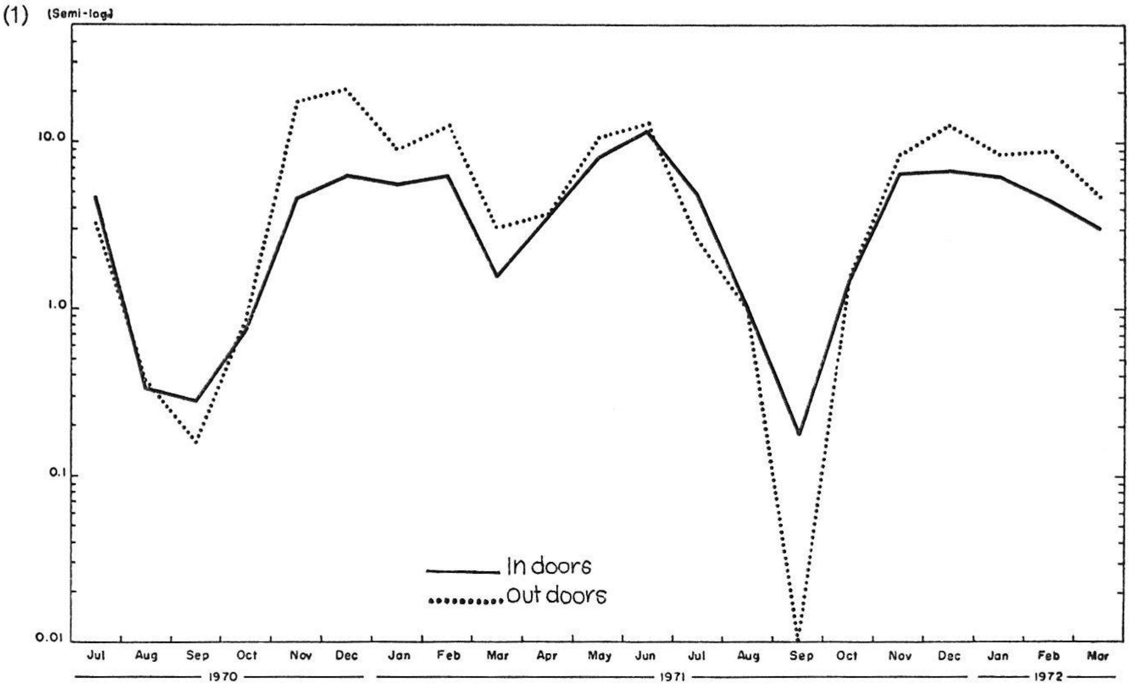
The difference in the endophagic and exophagic tendencies of *A. b. balabacensis* throughout the two seasons of observations could have been influenced by two main factors: rainfall and site of indoor collection. To elucidate this, the data were grouped for two periods: the first is from July 1970 to May 1971, when the indoor collections were carried out inside the large house (HQ laboratory), and the second is from June 1971 to March 1972 when the indoor collections were carried out inside a small hut (X). The data for each period were subgrouped according to the amount of rainfall recorded during the nights of collection, Table (2).

The data presented in Table 2 show that during the dry nights indoor biting of *A. b. balabacensis* tended to be more pronounced in the large house than in the small hut. During wet nights the tendency of indoor biting increased with the increase in the amount of rainfall but this may be more marked inside the large house than in the small hut.

*Table 2. A. b. balabacensis* collected on human baits indoors and outdoors during dry and wet nights in two periods of observations, July 1970 to May 1971 and June 1971 to March 1972

| Period | Site of indoor collections | Amount of rainfall from 18.00 to 06.00 hours | | | | | | | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | dry | | 0.1–10 mm | | 10.1–20 mm | | > 20 mm | | | |
| | | No. collected | | No. collected | | No. collected | | No. collected | | No. collected | |
| | | indoors | outdoors | indoors | outdoors | indoors | outdoors | indoors | outdoors | indoors | outdoors |
| July 1970 to May 1971 | large house | 1,001 (54.5) | 837 (45.5) | 706 (71.9) | 276 (28.1) | 476 (79.6) | 122 (20.4) | 528 (82.1) | 115 (17.9) | 2,711 (66.8) | 1,350 (33.2) |
| June 1971 to March 1972 | small hut | 721 (45.8) | 855 (54.2) | 763 (51.8) | 710 (48.2) | 149 (58.7) | 105 (41.3) | 185 (69.8) | 80 (30.2) | 1,818 (51.0) | 1,750 (49.0) |

Figures in parenthesis represent percentage of totals collected.




(1) Average no. of bites per man/night

*Fig. 5.* Man-vector contact in *A. minimus,* indoors and outdoors.

It appears that in general *A. b. balabacensis* will readily bite man indoors as well as outdoors. Well constructed houses with more inhabitants seem to be preferred to small huts with a lesser number of people. Also rain seems to encourage mosquitoes to enter houses or huts, most probably for shelter; this consequently leads to an increase in indoor biting. In Jamaica, THOMSON & MERCIER (1952) reported that *A. albimanus* was biting mostly outdoors during the dry season, while it had the tendency to bite more indoors during the rainy season. *A. albimanus* is an early biter and the authors concluded that the species changed its behaviour according to the habits of the human population which during the dry season spent the first hours of the night mostly outdoors, while during the rainy season the people entered the houses early at night. With regard to *A. b. balabacensis* such an explanation does not apply since the species is not an early biter, as will be shown later.

In our studies the results with *A. minimus* were different from those with *A. b. balabacensis,* Fig. 5. *A. minimus* tended to bite man more frequently outdoors than indoors. During the dry period the biting densities outdoors were generally 3–4 times higher than those observed indoors. When comparing the results of the season of high density from November to February in 1970/71 with those of the same period in 1971/72 it was noted that the outdoor biting tendency of *A. minimus* was more pronounced during the first season. In order to explain the

*Table 3. A. minimus* collected on human baits indoors and outdoors, during dry and wet nights in two periods of observations, July 1970 to May 1971 and June 1971 to March 1972

| Period | Site of indoor collections | Amount of rainfall from 18.00 to 06.00 hours | | | | | | Total | |
|---|---|---|---|---|---|---|---|---|---|
| | | dry | | 0.1–5 mm | | > 5 mm | | | |
| | | No. collected | | No. collected | | No. collected | | No. collected | |
| | | indoors | outdoors | indoors | outdoors | indoors | outdoors | indoors | outdoors |
| July 1970 to May 1971 | large house | 581 (31.4) | 1,270 (68.6) | 101 (37.8) | 166 (62.2) | 71 (55.5) | 57 (44.5) | 753 (33.5) | 1,493 (66.5) |
| June 1971 to March 1972 | small hut | 909 (40.4) | 1,340 (59.6) | 132 (47.5) | 146 (52.5) | 94 (70.2) | 40 (29.8) | 1,135 (42.7) | 1,526 (57.3) |

Figures in parenthesis represent percentage of totals collected.

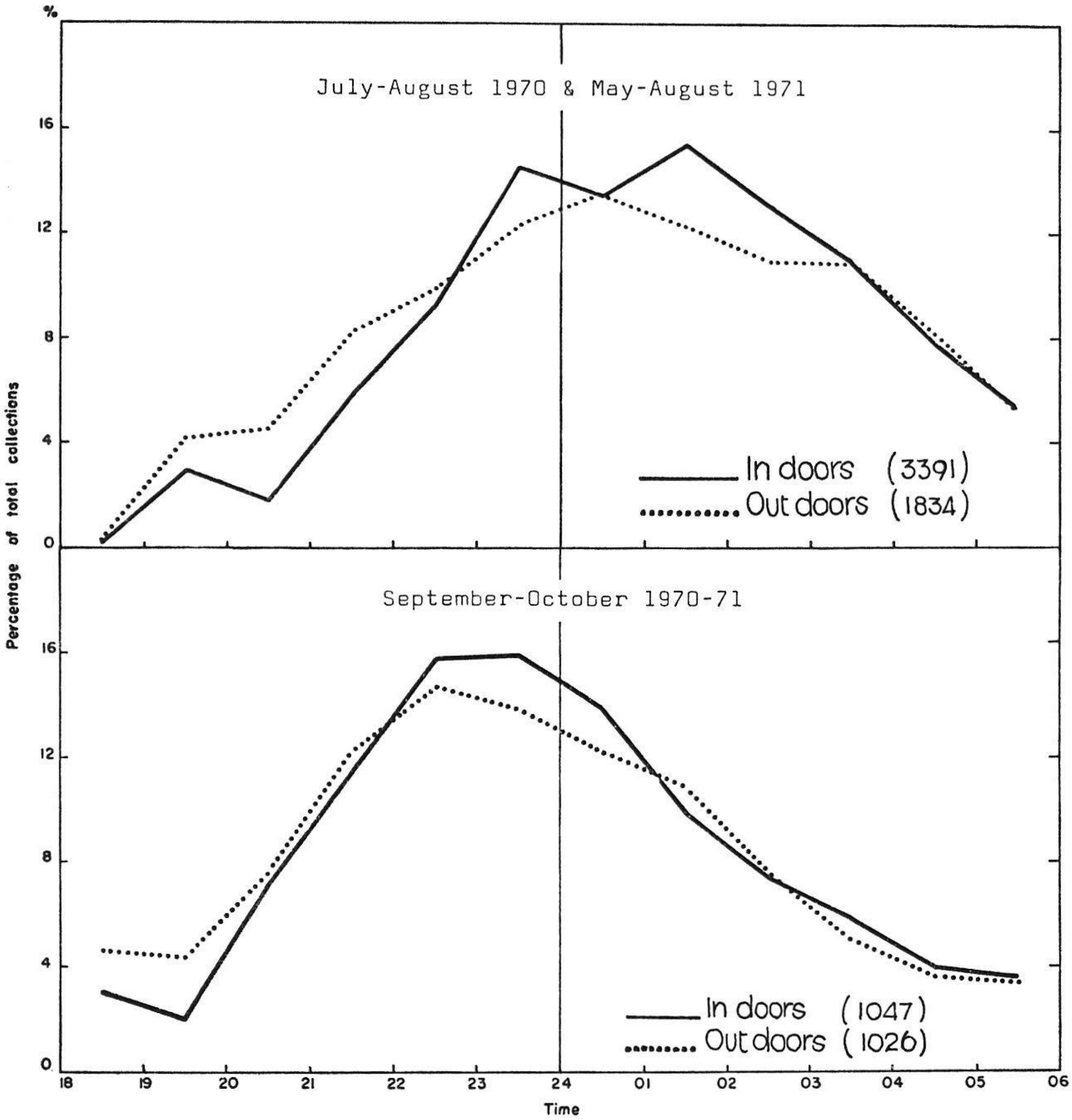
cause for this variation the data were grouped according to the site of collection and amount of rainfall, as previously done with *A. b. balabacensis*. However, only 3 divisions were made as the period of rainfall during the season of *A. minimus* was rather short.

The results in Table 3 seem to indicate that *A. minimus* tended to bite more inside the small hut which represents a more open type of structure than in the large house, with the reservation that the comparative observations in the two types of structures were not conducted during the same period. As in the case of *A. b. balabacensis*, the trend for its biting indoors increased with the increase in rainfall.

#### 4.1.3 Biting cycle

Variations were noted in the biting cycle of *A. b. balabacensis* and *A. minimus*. After examination of data, it has been found convenient to present the results for two periods: May–August and September–October for *A. b. balabacensis*, and May–August (wet season) and November–February (dry season) for *A. minimus*.

Results with *A. b. balabacensis* during May/August and September/October are illustrated in Fig. 6. During the former period indoor biting was very scarce in the first hour (18.00–19.00) and then increased gradually with the progress of the night. The biting activity reached its peak between 23.00 and 02.00 hrs and then gradually decreased until 06.00 hrs. After 06.00 hrs biting became very scarce and the results of a few other observations confirmed this finding. A similar pattern of biting activity was recorded outdoors but the peak was less sharp. For simplification, the biting activities may be considered for the

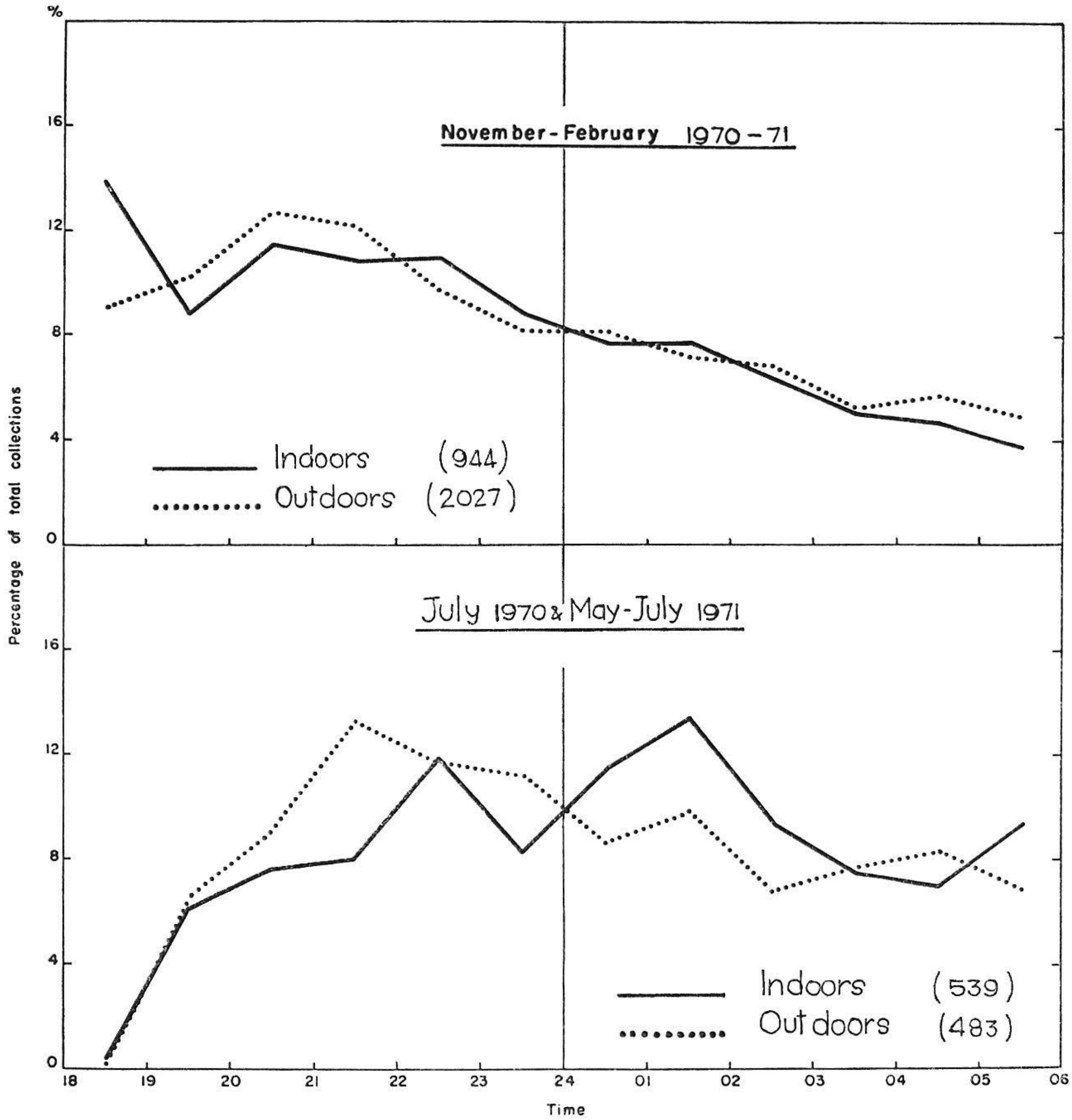



Note: Figures in parenthesis represent number of mosquitoes collected

*Fig. 6.* Indoor and outdoor biting time of *A. b. balabacensis.*

four quarters of the night. The highest biting density was recorded during the third quarter of the night. In September/October the indoor and outdoor biting of *A. b. balabacencis* started earlier than during the first period and became less during the late hours of the night. Also the peak of biting advanced and was now between 22.00 and 24.00 hrs, i.e. during the second quarter of the night. The patterns of biting activities indoors and outdoors were similar.

These results indicate that *A. b. balabacensis* is a late biter during its maximum breeding season, with a tendency to earlier biting towards the end of the season. Temperature seems to be the most important factor responsible for the seasonal variation in the biting cycle of *A. b. balabacensis.* The weather became cooler towards the end of the




Note: Figures in parenthesis represent number of mosquitoes collected

*Fig. 7.* Indoor and outdoor biting time of *A. minimus.*

season and the lowest temperatures were recorded during the second half of the night. The time of sunset and sunrise may also have some influence as there is a difference of 60 and 23 minutes, respectively, between the beginning of May and the end of October.

Results with *A. minimus* during the dry and wet seasons are illustrated in Fig. 7. During the dry season, from November to February, *A. minimus* used to bite indoors and outdoors throughout the night. It started with high biting density indoors during the first hour (18.00 to 19.00) and remained at a rather high level from 20.00 to 23.00 hrs. Outdoors, there was a considerable amount of biting in the first two hours, followed by a peak from 20.00 to 22.00 hrs. Biting indoors and outdoors gradually decreased towards the late hours of the night. Considering the four quarters of the night, the highest biting activity was observed during the first and second quarters, with nearly equal proportions. The activity was less during the third quarter, followed by the fourth. During the wet season, May/July, the results were different as biting started in the second hour and took place throughout the night with irregular peaks. The pattern indoors was different from that outdoors. Two marked peaks occurred during 22.00–23.00 hrs and 01.00–02.00 hrs indoors in addition to a smaller peak at 05.00 to 06.00 hrs, while outdoors there was one marked peak at 21.00 to 22.00 hrs. The highest biting rate indoors was recorded during the third quarter, whereas the highest biting rate outdoors was during the second quarter.

The above results indicate that *A. minimus* is an early biter during the dry season and a late biter during the wet season. As explained earlier with *A. b. balabacensis,* temperature seems to be the main factor responsible for this variation, whereas the time of sunset and sunrise may be a secondary factor.

#### 4.1.4 Age composition

The results of dissections for age composition in populations of *A. b. balabacensis* and *A. minimus* are presented in Tables 4A and B, respectively. Results with the former species are presented on a monthly basis with the exception of the data for October and November 1970, which were combined due to the relatively small number of specimen. On the other hand, results with *A. minimus* are given for two subsequent months each, so as to present larger samples. In general no marked variation of the parous rate indoors or outdoors was noted in *A. b. balabacensis* and *A. minimus*. Further analysis of data according to the four quarters of the night has failed to show any significant difference of the results obtained in both species.

The parous rate in *A. b. balabacensis* showed an increasing trend towards the end of the season in 1970. In 1971, when observations covered the full season of the species, the parous rate was high with 76.4 % at the beginning of the season in May, decreased in June, July and August to a level of 42–47 % and increased again in September and October reaching about 69 %. There was a significant difference between the parous rates recorded during August 1970 and September 1970 ($\chi^2 = 7.496$, $p < 0.01$), September 1970 and October/November 1970 ($\chi^2 = 17.358$, $p < 0.01$). In 1971, there were significant differences too in the parous rates between May and June and August and September ($\chi^2 = 64.307$, $p < 0.01$) ($\chi^2 = 64.960$, $p < 0.01$), respectively.

Based on the parous figures and on the assumptions that the feeding interval of *A. b. balabacensis* is 3 or 4 days and that one blood meal

*Table 4.* A – Age composition of *A. b. balabacensis*

| Month and year | Indoors | | | Outdoors | | | Total | | | Probability of daily survival * |
|---|---|---|---|---|---|---|---|---|---|---|
| | total dissected | parous No. | % | total dissected | parous No. | % | total dissected | parous No. | % | |
| July 1970 | 1,130 | 476 | 42.1 | 299 | 110 | 36.8 | 1,429 | 586 | 41.0 | 0.81 |
| Aug. 1970 | 464 | 190 | 41.0 | 124 | 52 | 41.9 | 588 | 242 | 41.2 | 0.81 |
| Sep. 1970 | 272 | 138 | 50.7 | 181 | 87 | 48.1 | 453 | 225 | 49.7 | 0.845 |
| Oct./Nov. 1970 | 281 | 189 | 67.3 | 334 | 195 | 58.4 | 615 | 384 | 62.4 | 0.89 |
| Total | 2,147 | 993 | 46.3 | 938 | 444 | 47.3 | 3,085 | 1,437 | 46.6 | 0.835 |
| May 1971 | 157 | 114 | 72.6 | 178 | 142 | 79.8 | 335 | 256 | 76.4 | 0.935 |
| June 1971 | 145 | 69 | 47.6 | 177 | 79 | 44.6 | 322 | 148 | 46.0 | 0.835 |
| July 1971 | 676 | 277 | 41.0 | 515 | 225 | 43.7 | 1,191 | 502 | 42.2 | 0.82 |
| Aug. 1971 | 326 | 146 | 44.8 | 280 | 138 | 49.3 | 606 | 284 | 46.9 | 0.835 |
| Sep. 1971 | 325 | 219 | 67.4 | 403 | 281 | 69.7 | 728 | 500 | 68.7 | 0.91 |
| Oct. 1971 | 102 | 67 | 65.7 | 150 | 106 | 70.7 | 252 | 173 | 68.7 | 0.91 |
| Total | 1,731 | 892 | 51.5 | 1,703 | 971 | 57.0 | 3,434 | 1,863 | 54.3 | 0.865 |

* The probability of daily survivals were derived from figure 4 in GARRETT-JONES & GRAB (1964).

B – Age composition of *A. minimus*

| Month and year | Indoors | | | Outdoors | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|
| | total dissected | parous No. | % | total dissected | parous No. | % | total dissected | parous No. | % |
| Nov./Dec. 1970 | 208 | 130 | 62.5 | 719 | 378 | 52.6 | 927 | 508 | 54.8 |
| Jan./Feb. 1971 | 137 | 79 | 57.7 | 268 | 146 | 54.5 | 404 | 225 | 55.6 |
| March/April 1971 | 64 | 43 | 67.2 | 78 | 54 | 69.2 | 142 | 97 | 68.3 |
| May/June 1971 | 114 | 64 | 56.1 | 221 | 106 | 48.0 | 335 | 170 | 50.7 |
| July/Aug. 1971 | 149 | 72 | 48.3 | 94 | 45 | 47.9 | 243 | 117 | 48.1 |
| Total | 672 | 388 | 57.7 | 1,380 | 729 | 52.8 | 2,052 | 1,117 | 54.4 |
| Nov./Dec. 1971 | 366 | 280 | 76.5 | 582 | 401 | 68.9 | 948 | 681 | 71.8 |
| Jan./Feb. 1972 | 218 | 141 | 64.7 | 373 | 226 | 60.6 | 591 | 367 | 62.1 |

sufficient for the production of the first batch of eggs (SLOOFF & VERDRAGER, 1972), the probability of daily survival was calculated from the graphs devloped by GARRETT-JONES and GRAB (1964), as shown in Table 4A. In the observations on *A. b. balabacensis* in 1971 the probability of daily survival was the highest in May with 0.935, decreasing in June, July and August to 0.835, 0.82 and 0.835, respectively. It increased again during the last two months of the season in September and October to 0.91.

Variations in the parous rates and probability of daily survival of *A. b. balabacensis* during its season were most likely due to the effect of rainfall and floods. The high parous rate observed in May could be a reflection of the preceding dry cool season conditions, after which period aged females make their appearance, attacking man early before the establishment of the new breeding places. During June–August, the extension of breeding places with the rains gave rise to high emergence resulting in an apparent increase in the proportion of the nulliparous females. In September when the parous rate was also high, floods had occurred and washed out most of the breeding places on the sides of the streams. This has apparently led to a decrease in the number of newly emerged females. This condition seemed to have continued throughout October.

During its season in 1970/71, *A. minimus* has shown similar parous rates in November/December with 54.8 % and January/February with 55.6 %. In March/April the parous rate increased to 68.3 %, dropping then to 50.7 % and 48.1% in May/June and July/August, respectively. The increase in March/April and the decrease in May/June appear to be significant ($\chi^2 = 7.062$ and 12.484, $p < 0.01$). The increase of the parous rate in March/April is probably due to partial dryness of the breeding places. The results of 1971/72 indicate an increasing trend of the parous rate as compared to the results in 1970/71, especially marked in the November/December periods when the rate reached 71.8 % against 54.8 %. It appears that the environmental conditions for the survival of *A. minimus* were more favourable in 1971/72 than in 1970/71.

During the seasons of prevalence of *A. minimus* there is a considerable variation in the climatic conditions: the rainy and hot season of May–October, the dry and cool season of November–February, and the dry and hot season of March–April, as shown in Fig. 3. It is to be expected that such climatic variations will affect the period of time between feeding and oviposition as the digestion of the blood meal and the maturation of the eggs take longer in cooler climates. THOMPSON (1941) observed in Assam, India, that *A. minimus* normally took two days to digest the blood meal during the monsoon season when the mean maximum and minimum temperature (July) ranged between

32.3 and 23.7 °C and the relative humidity was high. During the cool months with temperatures ranging between 23.3 °C and 10.3 °C (December) and with a high relative humidity, the period was extended to 4–6 days.

#### 4.1.5 Sporozoite infection

The results of gland dissections for sporozoites, since June 1971, are presented in Table 5.

Sixteen *A. b. balabacensis* and 3 *A. minimus* were found positive. The results show a monthly variation of the sporozoite rate in *A. b. balabacensis* between 0.16 % and 0.69 % during its season, with the highest rate in June, while among *A. minimus* there was only one positive each in June, November and February.

Previous records on the sporozoite rate in *A. b. balabacensis* in Thailand and elsewhere gave much higher values than the study area. SCANLON & SANDHINAND (1965) observed a sporozoite rate of 8.7 % in Cholburi Province in Thailand, MCARTHUR (1947) and CHANG (1958)[7] reported 2.4 % and 1.7 %, respectively, from Sabah in East Malaysia and CHEONG (1968) reported 3.0 % from West Malaysia. In Cambodia (Khmer), EYLES et al. (1964) observed rates of 2.4 % and 7.67 % in the villages and 0.6–1.0 % in forest areas. For *A. minimus* in Thailand, SCANLON & SANDHINAND (1965) quoted a higher rate (2.5 %) as well.

*Table 5.* Sporozoite infection in *A. b. balabacensis* and *A. minimus*

| Month and year | *A. b. balabacensis* | | | *A. minimus* | | |
|---|---|---|---|---|---|---|
| | total dissected | positive sporozoites | sporozoite rate | total dissected | positive sporozoites | sporozoite rate |
| June 1971 | 725 | 5 | 0.69 | 384 | 1 | 0.26 |
| July 1971 | 1,295 | 7 | 0.54 | 200 | 0 | – |
| Aug. 1971 | 612 | 1 | 0.16 | 60 | 0 | – |
| Sep. 1971 | 626 | 2 | 0.32 | 5 | 0 | – |
| Oct. 1971 | 270 | 1 | 0.37 | 58 | 0 | – |
| Nov. 1971 | 19 | 0 | – | 377 | 1 | 0.27 |
| Dec. 1971 | 7 | 0 | – | 534 | 0 | – |
| Jan. 1972 | 16 | 0 | – | 218 | 0 | – |
| Feb. 1972 | 2 | 0 | – | 301 | 1 | 0.33 |
| Mar. 1972 | 1 | 0 | – | 215 | 0 | – |
| Total | 3,573 | 16 | 0.45 | 2,352 | 3 | 0.13 |

[7] See footnote 6, page 133.

In the absence of insecticide spraying, the low sporozoite rates in *A. b. balabacensis* and *A. minimus* of the study area are most likely due to regular prophylaxis taken by all staff members and hired human baits and to the immediate radical treatment of malaria cases which has kept the parasite reservoir at a rather low level. In connection with these results it may be also noted that during the whole period of study no monkeys were observed in the study area or the neighbouring forest.

### 4.2 Outdoor daytime resting places

Search for outdoor daytime resting places in natural and artificial pit-shelters was carried out during the high density season of *A. b. balabacensis*. Only two specimens were encountered: an unfed one in a pit-shelter and a fed one in a tree hole.

### 4.3 House frequenting behaviour as studied by the use of experimental huts

Results with the two verandah-trap huts and the four portable-trap huts during 1971/72, with all the huts in operation and in their final design and location, are presented in Tables 6, 7 and 8. During that period the verandah-trap huts operated for 26 weeks from May to March and the portable-trap huts for 25 weeks from June to March. The time of operating the trap huts varied from 3–4 weeks per month to 1–2 weeks per month, depending on the circumstances. No observations were carried out in January. During the whole period a total of 1957 *A. b. balabacensis* and 221 *A. minimus* were recorded from the verandah-trap huts and 1586 *A. b. balabacensis* and 39 *A. minimus* from the portable-trap huts. With the former type of trap huts the average yield of *A. b. balabacensis* per trap hut week varied between 6 in October and 102.13 in July and of *A. minimus* between 1 in December and 25 in May while with the portable-trap huts the average yield of *A. b. balabacensis* varied between 5.25 in October and 47.92 in June and of *A. minimus* between 0.19 in August and 0.75 in November. From this it appears that the yield of both species was higher in the verandah-trap huts than in the portable-trap huts. With the verandah-trap hut offering more natural entry and exit to mosquitoes, and being larger in size and harbouring more baits, the above results are to be expected. Moreover, it was noted that the yield of *A. b. balabacensis* in the verandah-trap huts followed its seasonal prevalence, as shown by the indoor biting density per man/night, more closely than was the case with the portable-trap huts, Fig. 8.

The crude data obtained from the experimental huts show that the yield of *A. b. balabacensis* was much higher than that of *A. minimus*. A trial was made to examine the yield of the experimental huts versus

*Table 6.* Abdominal condition, egress and indoor resting with *A. b. balabacensis* and *A. minimus* from the verandah-trap huts during 1971/72

| Resting and Egress | Hut E | | | | | Hut F | | | | | Total E+F | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | fed | unfed | gravid | all stages | | fed | unfed | gravid | all stages | | fed | | unfed | | gravid no. | all stages | |
| | | | | No. | % D | | | | No. | % D | No. | % D | No. | % D | | No. | % D |
| Indoor resting | | | | | | | | | | | | | | | | | |
| *A. b. balaba.* | 36 | 4 | 0 | 40 | 4.0 | 30 | 3 | 0 | 33 | 3.4 | 66 | 3.5 | 7 | 8.6 | 0 | 73 | 3.7 |
| *A. minimus* | 8 | 0 | 0 | 8 | 7.0 | 5 | 1 | 1 | 7 | 6.6 | 13 | 6.9 | 1 | | 1 | 15 | 6.8 |
| Window traps | | | | | | | | | | | | | | | | | |
| *A. b. balaba.* | 112 | 10 | 0 | 122 | 12.3 | 126 | 4 | 0 | 130 | 13.6 | 238 | 12.8 | 14 | 17.3 | 0 | 252 | 12.9 |
| *A. minimus* | 5 | 0 | 0 | 5 | 4.3 | 6 | 1 | 0 | 7 | 6.6 | 11 | 5.8 | 1 | | 0 | 12 | 5.4 |
| Verandah traps | | | | | | | | | | | | | | | | | |
| *A. b. balaba.* | 800 | 30 | 6 | 836 | 83.7 | 762 | 30 | 4 | 796 | 83.0 | 1,562 | 83.7 | 60 | 74.1 | 10 | 1,632 | 83.4 |
| *A. minimus* | 90 | 6 | 6 | 102 | 88.7 | 74 | 16 | 2 | 92 | 86.8 | 164 | 87.3 | 22 | | 8 | 194 | 87.8 |
| Total | | | | | | | | | | | | | | | | | |
| *A. b. balaba.* | | | | | | | | | | | | | | | | | |
| collected | 948 | 44 | 6 | 998 | 100 | 918 | 37 | 4 | 959 | 100 | 1,866 | 100 | 81 | 100 | 10 | 1,957 | 100 |
| Stages % | 94.9 | 4.5 | 0.6 | 100 | | 95.7 | 3.9 | 0.4 | 100 | | 95.3 | | 4.3 | | 0.4 | 100 | |
| *A. minimus* | | | | | | | | | | | | | | | | | |
| collected | 103 | 6 | 6 | 115 | 100 | 85 | 18 | 3 | 106 | 100 | 188 | 100 | 24 | | 9 | 221 | 100 |
| Stages % | 89.6 | 5.2 | 5.2 | 100 | | 80.0 | 17.0 | 3.0 | 100 | | 85.0 | | 10.9 | | 4.1 | 100 | |

% D = % distribution.

*Table 7.* Abdominal condition, egress and indoor resting with *A. b. balabacensis* and *A. minimus* from portable-trap huts during 1971/72

| Resting and Egress | Hut A | | | | | Hut B | | | | | Hut C | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | All stages | | | | | All stages | | | | | All stages | |
| | F | Un | G | No. | % D | F | Un | G | No. | % D | F | Un | G | No. | % D |
| Indoor resting | | | | | | | | | | | | | | | |
| *A. b. balaba.* | 26 | 0 | 0 | 26 | 8.0 | 33 | 0 | 1 | 34 | 8.5 | 14 | 0 | 0 | 14 | 4.3 |
| *A. minimus* | 0 | 1 | 0 | 1 | | 2 | 0 | 0 | 2 | | 1 | 0 | 0 | 1 | |
| Window traps | | | | | | | | | | | | | | | |
| *A. b. balaba.* | 291 | 9 | 1 | 301 | 92.0 | 349 | 19 | 0 | 368 | 91.5 | 293 | 13 | 2 | 308 | 95.7 |
| *A. minimus* | 8 | 2 | 0 | 10 | | 4 | 1 | 1 | 6 | | 7 | 1 | 1 | 9 | |
| *Total* | | | | | | | | | | | | | | | |
| *A. b. balaba.* | | | | | | | | | | | | | | | |
| collected | 317 | 9 | 1 | 327 | 100 | 382 | 19 | 1 | 402 | 100 | 307 | 13 | 2 | 322 | 100 |
| Stages % | 96.9 | 2.8 | 0.3 | 100 | | 95.0 | 4.7 | 0.3 | 100 | | 95.4 | 4.0 | 0.6 | 100 | |
| *A. minimus* | 8 | 3 | 0 | 11 | | 6 | 1 | 1 | 8 | | 8 | 1 | 1 | 10 | |

F = fed; Un = unfed; G = gravid; % D = % distribution.

| Resting and Egress | Hut D | | | | | Total | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | All stages | | F | | Un | | | All stages | |
| | F | Un | G | No. | % D | No. | % D | No. | % D | G | No. | % D |
| Indoor resting | | | | | | | | | | | | |
| *A. b. balaba.* | 21 | 0 | 0 | 21 | 3.9 | 94 | 6.2 | 0 | | 1 | 95 | 6.0 |
| *A. minimus* | 0 | 0 | 0 | 0 | | 3 | | 1 | | 0 | 4 | |
| Window traps | | | | | | | | | | | | |
| *A. b. balaba.* | 481 | 30 | 3 | 514 | 96.1 | 1,414 | 93.8 | 71 | 100 | 6 | 1,491 | 94.0 |
| *A. minimus* | 4 | 1 | 5 | 10 | | 23 | | 5 | | 7 | 35 | |
| *Total* | | | | | | | | | | | | |
| *A. b. balaba.* | | | | | | | | | | | | |
| collected | 502 | 30 | 3 | 535 | 100 | 1,508 | 100 | 71 | 100 | 7 | 1,586 | 100 |
| Stages % | 93.8 | 5.6 | 0.6 | 100 | | 95.1 | | 4.5 | | 0.4 | 100 | |
| *A. minimus* | 4 | 1 | 5 | 10 | | 26 | | 6 | | 7 | 39 | |

*Table 8.* Number of *A. b. balabacensis* and *A. minimus* as recorded from the experimental huts during 1971/72

| Month and year | No. weekly obser-vations | Verandah trap huts E + F | | | | Portable trap huts A + B + C + D | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | *A. b. balabacensis* | | *A. minimus* | | *A. b. balabacensis* | | *A. minimus* | |
| | | No. | density * | No. | density * | No. | density * | No. | density * |
| May 1971 | 1 | 123 | 61.5 | 50 | 25.00 | – | – | – | – |
| June 1971 | 3 | 491 | 81.83 | 54 | 9.00 | 575 | 47.92 | 8 | 0.66 |
| July 1971 | 4 | 817 | 102.13 | 31 | 3.88 | 598 | 37.38 | 8 | 0.50 |
| Aug. 1971 | 4 | 303 | 37.91 | 23 | 2.88 | 188 | 11.75 | 3 | 0.19 |
| Sep. 1971 | 3 | 180 | 30.00 | 0 | 0.00 | 157 | 13.08 | 0 | 0.00 |
| Oct. 1971 | 3 | 36 | 6.00 | 7 | 1.17 | 63 | 5.25 | 6 | 0.50 |
| Nov. 1971 | 3 | 4 | 0.66 | 8 | 1.33 | 5 | 0.42 | 9 | 0.75 |
| Dec. 1971 | 1 | 0 | 0.00 | 2 | 1.00 | 0 | 0.00 | 1 | 0.25 |
| Jan. 1972 | – | – | – | – | – | – | – | – | – |
| Feb. 1972 | 2 | 3 | 0.75 | 27 | 6.75 | 0 | 0.00 | 2 | 0.25 |
| March 1972 | 2 | 0 | 0.00 | 19 | 4.75 | 0 | 0.00 | 2 | 0.25 |
| Total | 26 | 1,957 | – | 221 | – | 1,586 | – | 39 | – |

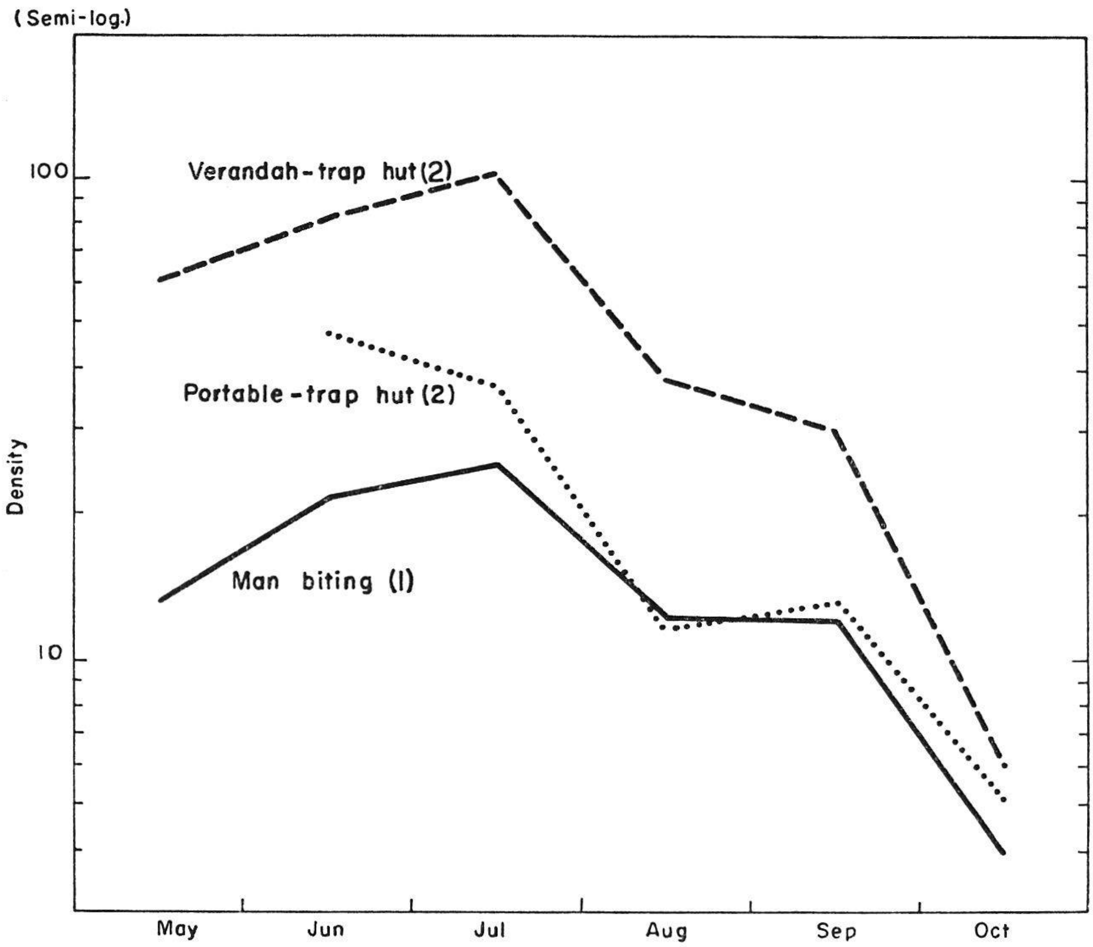
* Average number of mosquitoes recorded per trap hut/week.

the results obtained on man biting. The data of May and June 1971, when both species were prevalent in high density, were used for this purpose. The values per sleeper/night, with only blood-fed mosquitoes, were calculated from results of the verandah-trap huts and compared with those of indoor biting collections, per man/night (Table 9).

The data in Table 9 show that the relative prevalence of both species was at ratio of 1.8 : 1 *A. b. balabacensis* to *A. minimus* in the indoor biting collections and of 6.1 : 1 in the verandah-trap huts. When comparing the results obtained from both types of collection for each species the density of indoor biting was 6.7 and 25.0 times the yield of the verandah-trap huts in *A. b. balabacensis* and *A. minimus*, respectively. These results indicate that *A. b. balabacensis* enters rather compact structures with much less reluctance than *A. minimus*.

4.3.1 Verandah-trap huts

Detailed results with the verandah-trap huts are available for hut E in 1970 and for huts E and F in 1971/72. During the latter period, when both huts were in operation, nearly equal numbers of *A. b. balabacensis* were recorded from both huts, with 998 specimens from hut E and 959 specimens from hut F (Table 6). Also the results in respect to abdominal stages of the mosquitoes, way of egress through window traps or eaves and indoor resting, were similar in both huts. Data collected from both huts indicate that 96.3 % of the females left the huts before




Note: (1) Per man/night as recorded indoors
(2) Average yield per trap hut/week

*Fig. 8.* Average yield of trap huts with *A. b. balabacensis* versus result on man biting.

daytime, 83.4 % through the eaves and 12.9 % through the window traps. With fed females alone nearly the same proportions were obtained. From these results it can be concluded that *A. b. balabacensis* has a tendency to leave the huts before daytime and that it prefers egress through the eaves rather than the window traps. A high proportion, representing 95.3 % of the females recorded, had succeeded in taking a blood meal while only 4.3 % failed to do so. The number of gravid mosquitoes constituted only 0.4 % of the total. This very low number seems to indicate that, after having had a blood meal, the females spend all the period until oviposition outdoors. Results of 68 night observations during the period of August–October 1970, with the same species but only from one hut (E), show a similar pattern of behaviour as seen in Table 10.

In respect of the results with *A. minimus,* hut E yielded 115 and hut F 106 specimens in 1971/72. The combined data of both huts show

*Table 9.* Yield of verandah-trap huts versus results of indoor man biting collections May/June 1971

| Number of weeks | | Indoor biting | | | | Verandah-trap huts | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | *A. b. balaba.* | | *A. minimus* | | *A. b. balaba.* | | *A. minimus* | |
| indoor biting | verandah trap huts | No. | m/n * | No. | m/n * | No. | s/n ** | No. | s/n ** |
| 2 | 4 | 491 | 17.5 | 279 | 10 | 580 | 2.6 | 95 | 0.4 |

* Number per man/night.
** Yield per sleeper/night.

*Table 10.* Summary of observations with verandah-trap huts on *A. b. balabacensis* during 1970

| Distribution | Fed | | Unfed | | Gravid | | Total | |
|---|---|---|---|---|---|---|---|---|
| | No. | % D | No. | % D | No. | % D | No. | % D |
| Resting indoors | 25 | 4.4 | 1 | 2.9 | 0 | – | 26 | 4.3 |
| Window traps | 46 | 8.1 | 4 | 11.4 | 0 | – | 50 | 8.3 |
| Verandah-traps | 496 | 87.5 | 30 | 85.7 | 0 | – | 526 | 87.4 |
| Total No. | 567 | 100 | 35 | 100 | 0 | – | 602 | 100 |
| Stages % | (94.2) | – | (5.8) | – | – | – | (100) | – |

% D = % distribution.

that 93.2 % of all mosquitoes left the huts before daytime, 87.8 % through the eaves and 5.4 % through the window traps. The results in fed females alone were nearly the same. This indicates that the small population of *A. minimus* recorded from the verandah-trap huts behaved in a way similar to that of *A. b. balabacensis.* Mosquitoes left the huts before daytime and preferred to do so through the eaves rather than through the window traps. A high proportion, representing 85 % of all females recorded, succeeded to take blood meals during the same night whereas 10.9 % failed to do so. The proportion of *A. minimus* gravid females was 4.1%, thus higher than that observed in *A. b. balabacensis* (0.4 %).

#### 4.3.2 Portable-trap huts

Detailed results from the four portable-trap huts during 1971/72 are presented in Table 7. Hut D yielded the highest number of *A. b. bala-*

*Table 11.* Summary of observations with portable-trap huts on *A. b. balabacensis* during 1970

| Distribution | Fed | | Unfed | | Gravid | | Total | |
|---|---|---|---|---|---|---|---|---|
| | No. | % D | No. | % D | No. | % D | No. | % D |
| Resting indoors | 16 | 4.0 | 2 | 5.1 | 0 | – | 18 | 4.1 |
| Window traps | 381 | 96.0 | 37 | 94.9 | 0 | – | 418 | 95.9 |
| Total No. | 397 | 100 | 39 | 100 | 0 | – | 436 | 100 |
| Stages % | (91.1) | – | (8.9) | – | – | – | (100) | – |

% D = % distribution.

*bacensis* with 535 specimens, followed by hut B with 402 specimens, hut A with 327 specimens and hut C with 322 specimens. In analysing the data collected from the four huts it was noted that the results were in agreement with those obtained from the verandah-trap huts:

a. 94.0 % of all females left the huts before daytime and very similar results were obtained with fed females alone.
b. 95.1% of the females had taken a blood meal during the same night while only 4.5 % failed to do so, and 0.4 % had possibly fed in previous nights (gravid).

From July to October 1970 a total of 89 night observations were conducted using three portable-trap huts. They were of different material and design and scattered over a wide area. The results obtained during this period with *A. b. balabacensis* are in agreement with the above results as shown in Table 11.

With regard to *A. minimus* the portable-trap huts yielded only few specimens. The total number of mosquitoes collected from the four huts in 1971/72 was 39, of which 35 were collected from the window traps and only 4 were found resting indoors at 06.00 hrs. Examination of the abdominal condition revealed that 26 were recently fed, 6 unfed and 7 gravid. The number of *A. minimus* collected in 1970 was even less.

#### 4.3.3 Time of exodus

The exodus studies had the objective of finding out whether the mosquitoes rest indoors after having taken a blood meal, and if so, for how long. These observations were carried out by means of the outlet window traps installed in the portable-trap huts. Ideally such studies

*Table 12.* Time of exodus of *A. b. balabacensis* from the portable-trap huts versus hourly biting collections as recorded inside hut (X) during four weeks of observations in June/July 1971

| Time | Exit [1] | | Biting | | Time | Exit [1] | | Biting | |
|---|---|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | | No. | % | No. | % |
| 18.00–19.00 | – | – | 1 | 0.1 | 24.00–01.00 | 128 | 17.2 | 97 | 12.0 |
| 19.00–20.00 | 2 | 0.3 | 31 | 3.9 | 01.00–02.00 | 83 | 11.3 | 133 | 16.5 |
| 20.00–21.00 | 3 | 0.4 | 17 | 2.1 | 02.00–03.00 | 84 | 11.4 | 123 | 15.2 |
| 21.00–22.00 | 38 | 5.2 | 43 | 5.3 | 03.00–04.00 | 57 | 7.7 | 101 | 12.5 |
| 22.00–23.00 | 80 | 10.9 | 64 | 7.9 | 04.00–05.00 | 57 | 7.7 | 67 | 8.3 |
| 23.00–24.00 | 89 | 12.1 | 98 | 12.1 | 05.00–06.00 | 116 | 15.8 | 33 | 4.1 |
| – | – | – | – | – | Total | 737 | 100 | 808 | 100 |

[1] Figures only include blood-fed females collected from outlet window traps.

should have been conducted in local houses but these did not suit the purpose as their walls and floors are made of split bamboo, thus providing many openings for the escape of mosquitoes. Results on the time of exodus were obtained only in respect of *A. b. balabacensis* which was prevalent in high density and entered the huts more freely than any other species including *A. minimus*. For four weeks, two each in June and July, during the peak density of the species, mosquitoes were collected from the eight outlet window traps of the portable-trap huts on an hourly basis from 20.00 hrs until 06.00 hrs. Only freshly fed females were considered for the purpose of these studies. Fed females found resting indoors at 06.00 hrs were excluded from the results. These were 40 mosquitoes representing about 5% of the total collected. Results from the window traps for each hour have been calculated as percentages of the total and compared to the results of the night biting collections as recorded during the same periods inside hut (X). Data for both types of collection are presented in Table 12 and illustrated in Fig. 9.

The earlier peak of exit of *A. b. balabacensis,* which had already fed on baits in the trap huts, as compared with the peak of biting as recorded from direct bait capture, could not be easily explained. It is possible that the small, compact structures of the portable-trap huts with a concentration of passively exposed baits may have caused high attraction to mosquitoes, which left this artificial type of structure as soon as they had fed. Direct bait capture, however, was carried out in a less compact hut with larger openings, thus allowing mosquitoes to

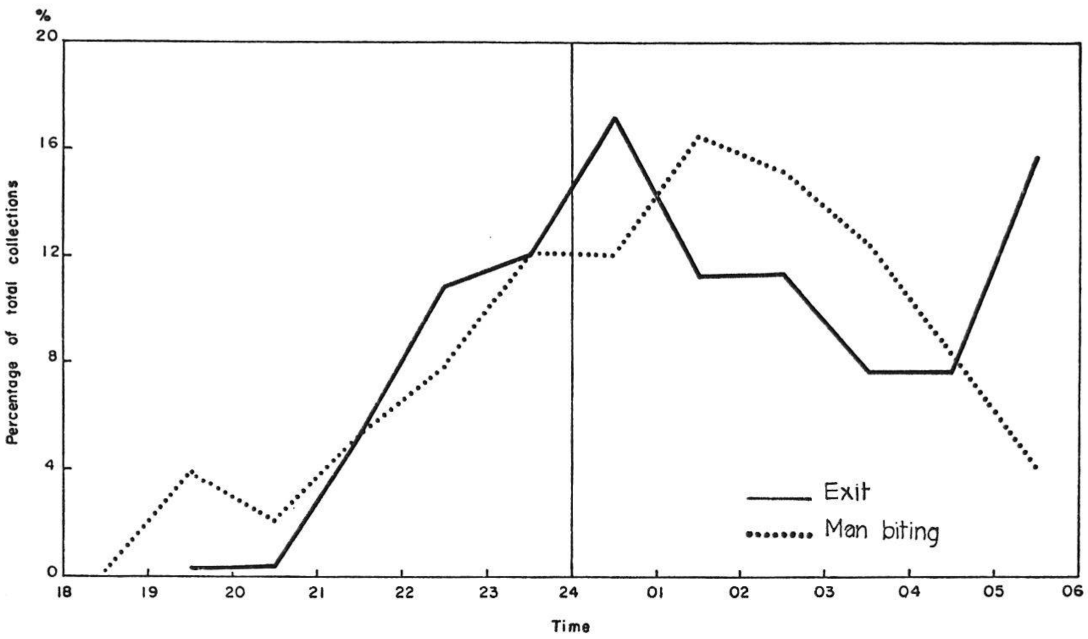



*Fig. 9.* Time of exit of blood-fed females *A. b. balabacensis* from portable trap huts versus result on its hourly biting collections.

follow a normal course of biting activity. It may also be that this difference is due to the variations in the two techniques since bait capture is carried out by collectors who were allowed ten minutes' break at the end of each hour and left their positions during this period. However, the impact of future spraying will be assessed by the two methods separately.

### 4.4 Epidemiology

During the repeated mass blood surveys and passive case detection activities, a total of 2,805 blood films were collected, out of which 252 were positive; 228 *P. falciparum* and 24 *P. vivax*. *P. falciparum* represented 90.5 % of the positives, out of which 32 % were with gametocytes.

Detailed results for one year observation, April 1971 to March 1972, are presented in Table 13.

The highest slide positivity rate was recorded during the period June–October 1971. During some months the number of positive films exceeded the number of malaria cases. This was due to repeated visits by very sick people to the PCD post at the field station and within short intervals seeking medical assistance. The persistence, or recurrence of symptoms and detection of the parasite in spite of adequate treatment is not unexpected in the study area. This is due to the known development of tolerance or resistance in *P. falciparum* to 4-amino-quinoline,

*Table 13.* Results of blood film examination and number of primary attacks by month, April 1971 to March 1972

| Month and year | No. slides collected | No. positives | Slide pos. rate (SPR) | Parasite | | | Primary attacks | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | *P. falciparum* | *P. falciparum* gametocytes | *P. vivax* | *P. falciparum* | *P. vivax* | total |
| Apr. 1971 | 126 | 2 | 1.6 | 0 | 0 | 2 | 0 | 2 | 2 |
| May 1971 | 134 | 3 | 2.2 | 3 | 0 | 0 | 2 | 0 | 2 |
| June 1971 | 152 | 28 | 18.4 | 22 | 6 | 0 | 20 | 0 | 20 |
| July 1971 | 182 | 35 | 19.2 | 28 | 5 | 2 | 18 | 2 | 20 |
| Aug. 1971 | 242 | 38 | 15.7 | 24 | 9 | 5 | 10 | 5 | 15 |
| Sep. 1971 | 232 | 46 | 19.8 | 30 | 16 | 0 | 12 | 0 | 12 |
| Oct. 1971 | 188 | 24 | 12.8 | 13 | 10 | 1 | 3 | 1 | 4 |
| Nov. 1971 | 161 | 9 | 5.6 | 4 | 3 | 2 | 2 | 2 | 4 |
| Dec. 1971 | 95 | 9 | 9.5 | 3 | 3 | 3 | 2 | 3 | 5 |
| Jan. 1972 | 104 | 8 | 7.7 | 6 | 1 | 1 | 1 | 1 | 2 |
| Feb. 1972 | 168 | 16 | 9.5 | 8 | 2 | 6 | 3 | 3 | 6 |
| March 1972 | 193 | 8 | 4.2 | 4 | 2 | 2 | 1 | 1 | 2 |
| Total (12 mo.) | 1,977 | 226 | 11.4 | 145 | 57 | 24 | 74 | 20 | 94 |
| Total * (21 mo.) | 2,805 | 252 | 9.0 | 155 | 73 | 24 | 90 | 20 | 110 |

* Total for the whole period of study, July 1970 to March 1972.

recrudescence after sulfadoxine treatment and possibly superinfection as well. Due to these complexity of factors, and in order to avoid vague conclusions, only the primary attacks were considered. The classification of primary attacks was based mainly on the following criteria:

a. Repeated negative results in the previous months, severe clinical symptoms and high density of the parasite in the absence of gametocytes.
b. Subsequent primary attacks are in cases which revealed clearance of the parasite for at least 6 months, followed by development of high fever with severe clinical symptoms and high parasite density. This criterium does not apply to vivax malaria and therefore no subsequent primary attacks of *P. vivax* were considered.

According to the above criteria, 110 attacks were primary and 142 attacks were relapse/recrudescence in a total of 93 persons. Seventy-seven persons had one single primary attack, 15 had 2 primary attacks, and only one revealed triple primary attacks. Ninety primary attacks were with *P. falciparum* and 20 with *P. vivax*.

The bulk of the cases (primary attacks) occurred during the period June–September 1971 (Table 13). A sharp rise with 20 cases was recorded in June. These cases must have contracted infection from the infected vectors during May and early June. The peak remained the same in July and then started decreasing in the following months. Nevertheless, a considerably high number of cases were recorded in August and September. The period of high transmission coincided fully with the season of *A. b. balabacensis* and partly with the wet season of *A. minimus*. During the dry season from November 1971 to March 1972 only few cases were detected every month. This result may indicate that transmission also took place during the dry season but at a low level, coinciding with the season of *A. minimus*.

In this study a trial was made to classify the malaria cases into 6 categories according to their place of residence and movement in relation to age (Table 14).

Data presented in Table 14 show that intensive infection took place in the field station, the neighbouring part of the village and inside the forest.

High positivity rates were noticed in all age groups. The lowest positivity rate with 15.4 % (2 cases) was detected from infants. In analysing the malaria cases according to sex and age it was noted that the positivity rate reached 30.3 % (76/251) among the males and 24.1 %

*Table 14.* Malaria cases (primary attacks) occurring in the study area by category, movement and age from July 1970 to March 1972

| No. | Category | Movement | Age group | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|
| | | | infant | 1–4 | 5–9 | 10–14 | 15–19 | 20 + | |
| 1 | Staff | stay in field station | 0 | 0 | 0 | 0 | 1 | 9 | 10 |
| 2 | Baits | stay in field station | 0 | 0 | 2 | 15 | 5 | 2 | 24 |
| 3 | Local villagers | stay in village | 2 | 6 | 7 | 0 | 2 | 10 | 27 |
| 4 | Local villagers | seasonally settle in forest | 0 | 1 | 0 | 0 | 2 | 11 | 14 |
| 5 | Villagers | indefinite movement | 0 | 2 | 4 | 2 | 1 | 10 | 19 |
| 6 | Outside villagers | seasonally settle in forest | 0 | 0 | 0 | 0 | 4 | 12 | 16 |
| Total number of malaria cases | | | 2 | 9 | 13 | 17 | 15 | 54 | 110 |
| Total number of people examined | | | 13 | 45 | 44 | 38 | 48 | 204 | 392 |
| Positivity rate % | | | 15.4 | 20.0 | 29.5 | 44.7 | 31.3 | 26.5 | 28.1 |

(34/141) among the females. Further analysis suggests that infections occurred more frequently among males of 10 years and more, indicating a higher exposure of this population group.

## 5. General discussion and conclusions

The studies conducted in the area of the forest fringe indicate that malaria transmission is perennial. The bulk of transmission occurs in the rainy season during which *A. b. balabacensis* is the predominant vector. Only later in the year, in the dry season, *A. minimus* becomes predominant. The latter species appears also in high density in the early part of the rainy season.

Bearing in mind that entomological and parasitological observations started in mid-1970 and that the number of people living around the fringe of the forest in the study area was rather limited, it is not astonishing that the number of malaria cases was less in 1970 as compared to 1971 when the observations covered a whole year and more people came to settle in the area.

Epidemiological and entomological findings, including the records of sporozoite rates, indicate that *A. b. balabacensis* is a more efficient vector than *A. minimus*. This confirms other studies in Thailand by GOULD et al. (1966). It should though be borne in mind that the lower night temperatures cause the sporogony to take more time during the dry season.

In the study area, *P. falciparum* is predominant, being present in 80–90% of all malaria cases. The remaining infections are due to *P. vivax*.

The observations showed that *A. b. balabacensis* bites man indoors and outdoors. Its contact with man is more intense in well-constructed human habitations than in forest huts. Rain at night seems to induce mosquitoes to bite more indoors. In contrast, *A. minimus* has shown less tendency to biting man indoors. Even in well-constructed huts, its biting density is much lower than that of *A. b. balabacensis,* although during the rainy nights likewise the indoor biting incidence increases. Observations with experimental huts indicate that the proportion of *A. minimus* caught in traps was much lower than in *A. b. balabacensis*. This may support the conclusion that under the prevailing conditions in the study area, *A. minimus* appears to be more exophagic than *A. b. balabacensis,* but both seem to be exophilic.

Results of previous observations in Thailand indicated that *A. minimus* is endophagic and endophilic and responded favourably to DDT house spraying (SAMBASIVAN et al., 1953)[8]. Workers elsewhere in the

[8] See footnote 5, page 130.

areas of its geographical distribution reported the same behaviour. It is possible that two biological variants of *A. minimus* exist, and that the domestic one became scarce with DDT house spraying and the present variant remained occupying the forest fringe, in the foothills, where conditions are suitable for its exophagic and exophilic behaviour, and favourable breeding sites are available. In its area of geographical distribution, *A. minimus* has been described as a domestic species (THOMSON, 1941, COVELL, 1944, and CHOW, 1970), but Chang (in COVELL, 1944) reported that the species exhibited an exophilic behaviour in South China.

With regard to the biting cycle, *A. b. balabacensis* is a late biter during most parts of its season. Inhabitants of the villages sleep in well-constructed huts while in the forest fringe or deep inside the forest they sleep in temporary huts, with thatched roofs and complete or incomplete walls made of split bamboo. During the rainy season, people retire early indoors, usually between 20.00 and 21.00 hours. Under these circumstances most of the contact of *A. b. balabacensis* with man occurs indoors during the late hours of the night. At the end of the rainy season, however, some early biting by *A. b. balabacensis* takes place. In contrast, *A. minimus* is an early biter during the dry season. During this period the people are indifferent in their habits: some stay late outdoors, gathering around fires for warmth, others enter dwellings. Thus there is the risk of being bitten indoors and outdoors during the dry season. During the rainy season *A. minimus* shows considerable biting activity outdoors shortly after sunset before people enter their dwellings, but the bulk of biting is delayed to later hours.

Observations with trap huts indicate that *A. b. balabacensis* enters rather compact structures in search of blood meals, but *A. minimus* is reluctant to do so. The verandah-trap hut offers advantages over the portable-trap hut since it gives a higher yield of *A. b. balabacensis*. On the other hand, the portable-trap hut is cheaper and easier to dismantle and move from one place to another. The results from the experimental huts have shown that *A. b. balabacensis* does not remain indoors for the completion of its gonotrophic cycle. About 95 % of *A. b. balabacensis* leave the huts after having taken a blood meal, preferably through the eaves rather than the window-traps. Early exodus soon after the blood meal is possible. These results are at variance with those of CHANG (1958)[9], in Sabah, where the majority of exodus occurred between 04.00 to 06.00 hours, indicating that a rather longer period is spent at rest indoors. Observations are going to continue after the area has been sprayed with DDT.

[9] See footnote 6, page 133.

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## *Zusammenfassung*

Im Programm der Malariaausmerzung in Thailand ergaben sich Schwierigkeiten bei der Unterbrechung der Übertragung in Wald- und Vorgebirgsgebieten, wo die Malariavektoren *A. b. balabacensis* and *A. minimus* vorherrschend sind. Deshalb wurden Feldstudien unternommen in einem typischen Waldrandgebiet und einer Vorgebirgsgegend im Norden Thailands zur Abklärung der Auswirkungen der Bekämpfungsmaßnahmen mit DDT-Depotbesprühungen auf die zwei Malariavektoren. Die Studie schloß auch Beobachtungen über andere epidemiologische Faktoren ein. Im ausgewählten Gebiet wurde eine Feldstation, ähnlich einem Dorfweiler, eingerichtet. Die Untersuchungen waren für zwei Perioden geplant, eine vor und eine nach der DDT-Depotbesprühung. Die Resultate der ersten Periode von 21 Monaten sind in dieser Publikation dargelegt. Die vorliegende Untersuchung erbrachte wichtige Befunde über die beiden Vektoren in bezug auf jahreszeitliche Verbreitung, Kontakte mit dem Menschen innerhalb und außerhalb des Hauses, Brutplätze, Stechzyklus, Alterszusammensetzung und Sporozoiteninfektion. Es wurden auch Versuche unternommen, die Tagesruheplätze zu lokalisieren. Das Verhalten der beiden Vektoren bei der Aufsuchung von Häusern wurde mit Hilfe von zwei verschiedenen durch Menschen geköderte Experimentierhütten studiert. *A. minimus* zeigte stärkere exophage und exophile Tendenzen als *A. b. balabacensis,* aber trotzdem war der letztere Vektor wirkungsvoller. Die Resultate über *A. minimus* stehen im Gegensatz zu früheren Befunden, die in der Ebene erhalten wurden. Massen-Blutuntersuchungen wurden monatlich durchgeführt. Blutausstriche wurden auch in der Feldstation gemacht, die damit ebenfalls als passive Krankheitsfundstelle diente. Alle Malariafälle wurden gründlich untersucht. Die Resultate haben einen Einblick gegeben über die endemische Verbreitung der Malaria, die Übertragungszeit, die vorherrschenden Malariaparasitenarten und über die Orte, wo die Infektion stattfand.

## *Résumé*

Le programme d'éradication du paludisme en Thailande s'est heurté à des difficultés pour interrompre la transmission dans les zones de forêts des contreforts montagneux où les vecteurs *A. b. balabacensis* et *A. minimus* étaient prédominants. En conséquence, des études sur la réponse de ces deux vecteurs vis-à-vis des pulvérisations résiduelles de DDT ont été entreprises dans une région typique de lisière de forêts et de contreforts montagneux dans le nord de la Thailande. Ces études comprenaient également des observations sur d'autres facteurs épidémiologiques. Une station d'étude, identique à un des villages de la région, a été établie dans un site sélectionné. Le plan des études a été divisé en deux périodes, une avant et une après les applications de DDT en pulvérisation résiduelle. Les résultats obtenus durant la première période, d'une durée de 21 mois, sont présentés dans ce papier. Ce travail a permis de mettre en évidence

d'importantes observations sur les deux vecteurs concernant la prévalence saisonnière, les contacts intérieurs et extérieurs avec l'homme les gîtes larvaires, le cycle d'agressivité, la courbe des âges et les taux de sporozoites. Des efforts ont également été entrepris pour localiser les gîtes de repos durant la journée. Les habitudes de fréquentation des maisons par les deux vecteurs ont été étudiées en utilisant deux différents types de hute constituant un piège expérimental. *A. minimus* a montré une plus forte tendance à l'exophilie et à l'exophagie que *A. b. balabacensis,* mais néanmoins ce dernier a été reconnu plus efficient. Les résultats concernant *A. minimus* sont différents des premières observations faites sur ce vecteur dans les plaines. Des enquêtes parasitologiques ont été réalisées à un mois d'intervalle. Les prélèvements de sang ont été récoltés à la station d'étude qui fonctionnait donc comme un poste de dépistage passif des cas de malaria. Ces derniers, après avoir été diagnostiqués, étaient investigués complètement. Les résultats de ces dernières observations ont permis d'éclaircir certains problèmes concernant l'endémicité de la malaria, la période de transmission, les espèces de parasites prédominants et les localités où l'infection avait lieu.