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9. ABSTRACT

Research objectives of the Central America Malaria Research Station (CAMRS) in El Salvador require means of measuring anophelism apart from commonly used domicile methods. Anopheles albimanus and A. pseudopunctipennis are present in large numbers in natural diurnal resting sites, and are less abundant in artificial sites. Favored natural sites are "rock-oriented" and include crevices of large and small rocks, rock walls, and lava deposits in shaded areas. Tree trunks, cavities, and roots also offer favorable sites. From natural sites, 181 collections yielded 7,670 A. albimanus and 2,344 A. pseudopunctipennis for an average of 42.4 and 12.9, respectively. Of these, the sex ratio of A. albimanus was 24.5 percent males and 75.5 percent females, while that of A. pseudopunctipennis was 34.0 percent males and 66.0 percent females. A. pseudopunctipennis seemed to prefer darker, more protected niches than A. albimanus. A. albimanus females moved into resting turned between midnight and 3 a.m., whereas A. pseudopunctipennis females were absent from resting sites throughout the period of darkness. The results emphasize the usefulness of diurnal resting sites for density measurements associated with control projects and biological studies, and as a source of specimens for experimental use.

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STUDIES ON THE DIURNAL RESTING HABITS OF *ANOPHELES ALBIMANUS* AND *A. PSEUDOPUNCTIPENNIS* IN EL SALVADOR¹

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ABSTRACT. This paper describes and quantitates the various types of diurnal resting sites available to and utilized by *A. albimanus* and *A. pseudopunctipennis* in El Salvador, discusses the 24-hour

resting patterns of the two species, and interprets the significance of the observations to the overall investigative activities of the Central America Malaria Research Station (CAMRS).

INTRODUCTION

The evaluation of area-wide techniques, e.g., ultra-low-volume (ULV) adulticiding and selective larviciding, as possible malaria eradication tools has required methods of measuring anophelism apart from the more commonly used indices associated with residual wall spraying of domiciles. This need led to a study of the daytime resting habits of *Anopheles albimanus* and *A. pseudopunctipennis*, the two most common anophelines in El Salvador. The former species is considered to be the principal vector of malaria in Central America and perhaps the only vector in El Salvador.

MATERIALS AND METHODS

Anophelines were first found on or under artificial structures such as bridge and culverts associated with pooled river water but many hours of diligent searching were required before the first anophelines were found in a completely natural situation, *viz.*, among dark and humid crevices of rocks along the shaded margins of a river bed. The discovery of the favored natural sites required many additional days and even weeks of searching.

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However, once the initial discoveries were made, mosquitoes were found at natural resting sites in every area of the country.

To determine the 24-hour resting pattern, two breeding foci, one each for *A. albimanus* (Playa Grande, near San Miguel) and *A. pseudopunctipennis* (Parras Lempa, near San Vicente), were selected on the basis of the presence of a variety of natural resting sites harboring large numbers of diurnally resting mosquitoes. The same procedure, outlined below, was followed at each location; at Playa Grande for *A. albimanus*, August 28-29, 1969, and at Parras Lempa for *A. pseudopunctipennis*, August 25-26, 1969.

Seven counting stations were established, one of which was completely natural, while the remaining six stations consisted of three red box shelters each. The boxes (1 ft. x 1 ft. x 1 ft. cube open on one side and painted red inside and out) were of the type described by Goodwin (1942), and served to make counting easier and more uniform. The boxes were placed in niches harboring mosquitoes resting on natural surfaces the afternoon before counts were to begin the following morning. A set of three boxes was used at each station to increase the chance of occupancy by mosquitoes. Counts of resting mosquitoes were made at 1-hour intervals over a 24-hour period by several teams with pre-planned work assignments. Counts were made at more frequent intervals during periods when mass movement was expected to occur. Results from all seven stations were combined since there was minimum variation from station to station. These experiments have been

repeated several times for each species in other localities and in different seasons.

RESULTS AND DISCUSSION

NATURAL DIURNAL RESTING. Results of specific collections, supplemented by continuing observations, have been used to characterize the favored natural diurnal resting sites of *A. albimanus* and *A. pseudopunctipennis* in El Salvador. In general, almost any shaded and protective crevice or shelter in areas where adults abound may serve as harborage for both species. However, clusters of large rocks in vegetated areas near breeding sites are

the most frequented niches, followed by rock walls, usually lava, associated with human housing or animal concentrations; small ground rocks in woodlands; shaded lava fields; tree trunks and cavities; and miscellaneous sites such as animal burrows and horizontal holes in the sides of shaded cliffs. Niches found just inside of the canopied portion of woodland-pasture ecotones or in fence rows seem preferred to those either deeper in woodlands or in isolated shelters within open areas.

A record of natural sites, listed by frequency of harborage of diurnally resting anophelines, along with brief descriptions and comments, is as follows:

Type of Site and Description	Comments
Large Rocks. Beneath and between large angled or flat rocks in shaded woodlands or woodland-field ecotones.	Excellent collecting situation— <i>A. albimanus</i> in more exposed sites; <i>A. pseudopunctipennis</i> in darker and more protected sites.
Rock Walls. Rock, stone, or lava walls near breeding sites, human habitation or animal concentrations.	Such walls are common in high density situations (around huts, cattle corrals, etc.) a good index situation.
Ground Holes. Protected ground holes (leaves and twigs) and associated rocks and tree depressions at ground level in shaded areas.	More common in soft ground associated with swamp or lowland conditions, particularly mangrove swamps.
Small Rocks and Tree Trunks. Beneath or among small rocks in shaded areas at ground level and among associated tree roots and trunks.	Offer numerous niches in shaded woodlands near breeding areas— <i>A. albimanus</i> in more exposed niches, <i>A. pseudopunctipennis</i> in more protected niches.
Tree Hollows and Roots. In cavities of large trees; between or among aboveground roots forming cavity-like recesses.	<i>A. albimanus</i> in more exposed sites. <i>A. pseudopunctipennis</i> in more protected sites.
Tree Trunks and Fallen Logs. Shaded outside of tree trunks and on underside of logs near ground. Shaded areas.	<i>A. albimanus</i> in rather exposed sites, e.g., a rotted stump in open field.
Small Rocks in Shaded Margins of Stream Beds. Beneath or among such rocks.	Offer scattered, isolated resting sites; require much searching, but probably an important dry season niche.
Lava Deposits. Large lava deposits in shaded areas, many large and small recesses.	Offer numerous niches. These lava beds were in banana groves near breeding (200–300 meters). Only <i>A. albimanus</i> , probably too much light for <i>A. pseudopunctipennis</i> .
Large Cliffside. Abrupt cliffside with moderate to heavy vegetative cover. Large and small rock outcroppings, cave-like recesses.	<i>A. albimanus</i> in more exposed sites; <i>A. pseudopunctipennis</i> in more protected sites.
<hr/>	
Miscellaneous Sites.	
Horizontal cliffside cavity behind waterfall.	Very dark, favors <i>A. pseudopunctipennis</i> .

Base of century plant.

Animal burrow (probably armadillo). Around mouth and in cavity.

Only *A. albimanus*, probably too exposed for *A. pseudopunctipennis*.

A high yield situation with a much greater concentration than other type niches in the same area. Mosquitoes around mouth and in neck of burrow.

A. pseudopunctipennis showed a preference for darker, more protected places than did *A. albimanus*. Both species seem to be "rock-oriented" in the selection of natural resting sites. Such sites are numerous in all of El Salvador and offer gradients of temperature, humidity, and light, as well as protection from wind and rain. Figure 1 shows natural situations

fall) while *A. pseudopunctipennis* was missing from lava deposits, tree trunks and fallen logs, animal burrows, and century plant collections. In some niches, it is believed that excessive light was the reason for the absence of *A. pseudopunctipennis*. The overall sex ratio of *A. albimanus* was 24.5 percent males and 75.5 percent females, that of *A. pseudo-*

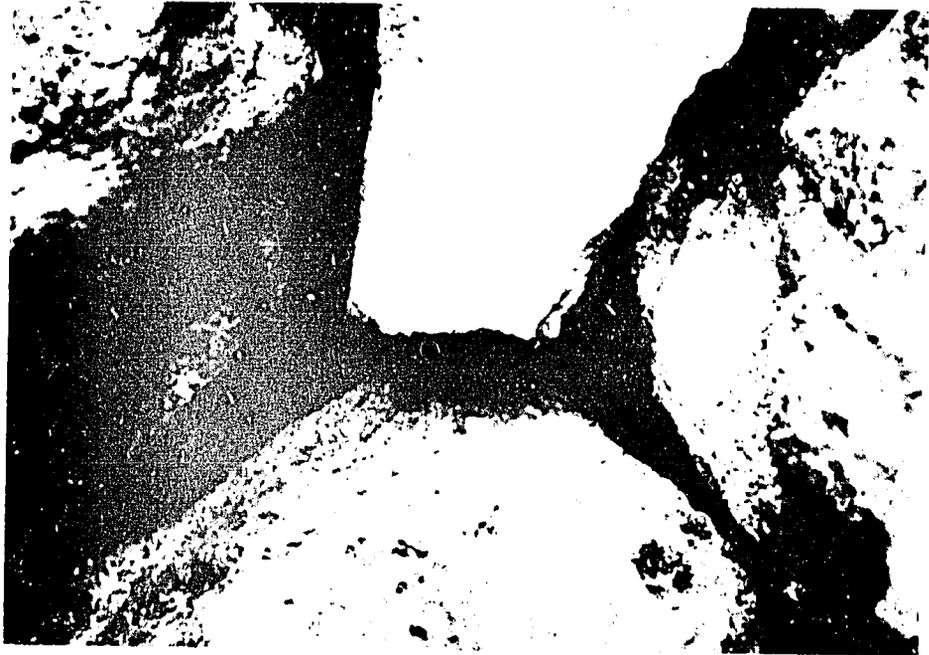


FIG. 1.—*A. albimanus* in typical natural diurnal resting site in rock crevice.

with resting mosquitoes which typify some of the sites described above.

The data in Table 1 show that the 181 specific collections of this study yielded 7,670 *A. albimanus* adults (76.6 percent) and 2,344 *A. pseudopunctipennis* adults (23.4 percent). *A. albimanus* was collected from all but one type site (behind water-

punctipennis, 34.0 percent males and 66.0 percent females. A general observation has been that the nearer the breeding area, the higher the percentage of males.

Characterization of natural resting sites of these anophelins has provided a valuable tool for indexing mosquito populations in connection with studies on

TABLE 1.—Summary of captures of diurnally resting adult anophelines from natural situations, El Salvador, February 1968–June 1970.

Type of Site	No. of Coll.	<i>A. albimanus</i>				<i>A. pseudopunctipennis</i>				Both Species			
		M.	F.	Tot.	Avg.	M.	F.	Tot.	Avg.	M.	F.	Tot.	Avg.
Large Rocks	104	1184	2526	3710	35.7	707	1257	1964	18.8	1891	3783	5674	54.6
Rock Walls	27	318	2441	2759	102.1	0	185	185	6.9	318	2626	2944	109.0
Small Rocks and Tree Trunks	11	100	317	417	37.9	4	48	52	4.7	104	365	469	42.6
Tree Hollows and Roots	10	31	40	71	7.1	23	10	33	3.3	54	50	104	10.4
Ground Holes	13	115	166	281	21.6	37	29	66	5.1	152	195	347	26.7
Lava Deposits	2	63	189	252	126.0	0	0	0	0	63	189	252	126.0
Large Shaded Cliffside	2	9	9	18	9.0	8	3	11	5.5	17	12	29	14.5
Tree Trunks and Fallen Logs	6	24	18	42	7.0	0	0	0	0	24	18	42	7.0
Small Rocks, Shaded Margins of Stream Beds	3	0	12	12	4.0	3	0	3	1.0	3	12	15	5.0
Miscellaneous Sites													
Horizontal cliffside cavity behind waterfall	1	0	0	0	0	16	14	30	30.0	16	14	30	30.0
Base of Century Plant	1	3	14	17	17.0	0	0	0	0	3	14	17	17.0
Animal Burrow	1	34	57	91	91.0	0	0	0	0	34	57	91	91.0
Total	181	1881	5789	7670	42.4	798	1546	2344	12.9	2679	7335	10014	55.3
		(24.5%)	(75.5%)			(34.0%)	(66.0%)			(26.8%)	(73.2%)		

bionomics and control, and has proved to be a good source of specimens for experimental work both in the field and laboratory. The collection of specimens from diurnal resting sites for host preference studies, for example, would seem to result in less bias than collections from nighttime host-specific situations such as cattle corrals. A good example of the value of using daytime resting situations for density measurements and as a source of specimens for epidemiological studies is offered by our experience with *A. pseudopunctipennis*. Adults of this species are not well represented in any of the commonly used night collecting methods, i.e., stable captures, human bait, light traps, or domicile collections; even in areas where larval surveys indicate a preponderance of this species in breeding areas. However they are often present in diurnal shelters.

For *A. albimanus*, counts from natural diurnal resting places near breeding areas probably reflect adult populations as well as some of the more commonly used night collection methods. Experience has shown that when *A. albimanus* is found during night captures, it is correspondingly present in daytime niches in the same vicinity and much more conveniently counted. Recent observations (July 9, 1970) near Lake Jocotal showed, for example, approximately 2,000 *A. albimanus* females counted during the day in the vicinity of a cattle corral. The count was made in 20-30 minutes and could have been made during any hour of the day. The same pattern applied to natural resting sites near the corral at Playa Grande and at a third location near Jocotal. This method has been used at many corral sites throughout El Salvador with good results. Continuing counts from natural resting sites in the vicinity of Estero Ticuiziapa, near La Libertad, revealed the same seasonal peaks as the light trap data for the same place and period.

DIURNAL RESTING IN ARTIFICIAL STRUCTURES. Early CAMRS studies indicated that such man-made structures as bridges and culverts were used by both *A.*

albimanus and *A. pseudopunctipennis* as diurnal resting shelters. Since this is true of some common North American anophelines which also use places for shelter, it was suspected that these two species might respond similarly to portable artificial shelters, such as red boxes. Although these devices have been used only to a limited extent, results so far indicate the validity of the assumption. In a high density situation, as many as 200 *A. albimanus* have been caught in a single red box (Lake Jocotal, near San Miguel); in a low density situation, involving mostly *A. pseudopunctipennis* (Pantano Ticuiziapa, near La Libertad), three red boxes used for a 3-day period performed very well (Table 2). Additionally, red boxes have been used with good results in connection with studies on the 24-hour resting pattern of *A. albimanus* and *A. pseudopunctipennis* as described in the materials and methods section of this report.

Bridges are especially good daytime resting places during the dry season when breeding takes place in the pooled water of the rivers below. Since rivers are a major dry season breeding source, and in some areas the principal source, much use can be made of bridge counts to reflect seasonal population changes and to evaluate results of control projects involving the rivers. The data of Table 3 show results obtained from bridge counts over four rivers in the La Libertad area of El Salvador. The data show a preponderance of *A. pseudopunctipennis* in two of the rivers (Jute and Huiza) which accurately reflect larval breeding recorded for the same period. Rio Aquisquillo, on the other hand, shows a preponderance of *A. albimanus*, although *A. pseudopunctipennis* dominated larval samples. This is attributed to the fact that the bridge over Aquisquillo is higher and permits more light than the others and thus inhibits *A. pseudopunctipennis* resting. The bridge over Rio San Antonio reflected the predominance of *A. albimanus* breeding in the associated river. Culverts are relatively rare and are not sufficiently dependable to be very useful as density index sites.

TABLE 2.—Summary of anopheline collections from three red boxes, artificial shelters, Pantano Ticuiziapa, La Libertad, El Salvador, June 18–20, 1968.

Box number *	Date	<i>A. albimanus</i>			<i>A. pseudopunctipennis</i>			Both species		
		Male	Female	Total	Male	Female	Total	Male	Female	Total
1	June 18	0	2	2	15	8	23	15	10	25
	June 19	0	0	0	16	8	24	16	8	24
	June 20	0	0	0	23	17	40	23	17	40
Total		0	2	2	54	33	87	54	35	89
2	June 18	2	1	3	0	0	0	2	1	3
	June 19	0	0	0	0	0	0	0	0	0
	June 20	0	0	0	5	0	5	5	0	5
Total		2	1	3	5	0	5	7	1	8
3	June 18	1	1	2	3	0	3	4	1	5
	June 19	0	0	0	16	6	22	16	6	22
	June 20	0	0	0	7	3	10	7	3	10
Total		1	1	2	26	9	35	27	10	37
Grand Total 3 boxes 3 days		3	4	7	85	42	127	88	46	134

* Box No. 1 Situated in a hillside north side of coastal marsh, beneath a large root and rock outcropping under rather dense vegetation along a fence row, box at ground level with opening facing south.

Box No. 2 Situated in a woodland on north side of coastal marsh, box at ground level under dense foliage opening facing west.

Box No. 3 Situated under a large slanted rock in small woodland on north side of coastal marsh, heavily shaded, box opening facing southeast.

Outbuildings tend to be of loose structure and are not sufficiently abundant to offer many sites for diurnally resting anophelines. However, occasionally one finds an outbuilding of brick or masonry construction which offers ideal harborage. On July 7, 1970, such a structure approximately 15' x 5', near the establo at Lake Jocotal yielded several thousand blood-fed females of *A. albimanus*. Similar counts

have been recorded from the same shelter on other occasions. Perhaps the development of a masonry or clay-type artificial shelter is indicated.

THE 24-HOUR RESTING PATTERN. Upon learning the daytime whereabouts of the two common anopheline species, it remained to determine their 24-hour pattern of movement into and out of diurnal resting situations. The resting pattern among

TABLE 3.—Summary of *A. albimanus* and *A. pseudopunctipennis* adults in daytime bridge collections, La Liberated area of El Salvador January–June, 1970.

Bridge	No. col.	<i>A. albimanus</i>				<i>A. pseudopunctipennis</i>			
		Males	Females	Total	Ave.	Males	Females	Total	Ave.
Jute	15	22	65	87	5.8	194	141	335	22.3
San Antonio *	14	45	48	93	6.6	5	7	12	0.9
Acquisquillo *	16	15	123	138	8.6	4	11	15	0.9
Huiza	19	91	176	267	14.0	391	773	1164	61.3
Total	64	173	412	585	9.1	594	932	1526	23.8

* Bridges over San Antonio and Acquisquillo Rivers permit more light and are not favorable to *pseudopunctipennis* resting.

females differed between *A. albimanus* and *A. pseudopunctipennis*. The pattern was similar for males of the two species which were present in resting sites between dawn and dusk and absent between dusk and dawn. The vast majority of resting females of both species were blooded. The diel resting cycle of females of *A. albimanus* and *A. pseudopunctipennis* is described below and diagrammed in Figure 2.

A. albimanus. The resting population of females was stable from 12:00 noon to 4:00 pm when the mosquitoes became restless and a little movement began. Mass migration out of the resting places occurred between 6:00 pm and 6:30 pm. During the early evening hours immediately after darkness, there was little or no resting. Between 8:00 pm and 12:00 pm there was minimum movement into the natural

resting sites, but there was steady and increasing movement into such places between 12:00 midnight and 2:00 am. Between 2:00 am and 5:00 am the population in natural resting places reached a peak. Between 5:00 am and 6:00 am there was some "resettling" followed by a stabilization in numbers in a given place until around 9:00 am after which numbers remained essentially constant until late afternoon.

A. pseudopunctipennis. The resting population of females was stable between 12:00 noon and 5:00 pm. At about 5:00 pm the specimens became restless, but mass migration out of the diurnal resting sites took place between 6:00 pm and 6:30 pm. During the darkness of night (6:30 pm-5:00 am) no mosquitoes were found in the natural resting or box stations. Between 5:00 am and 6:00 am

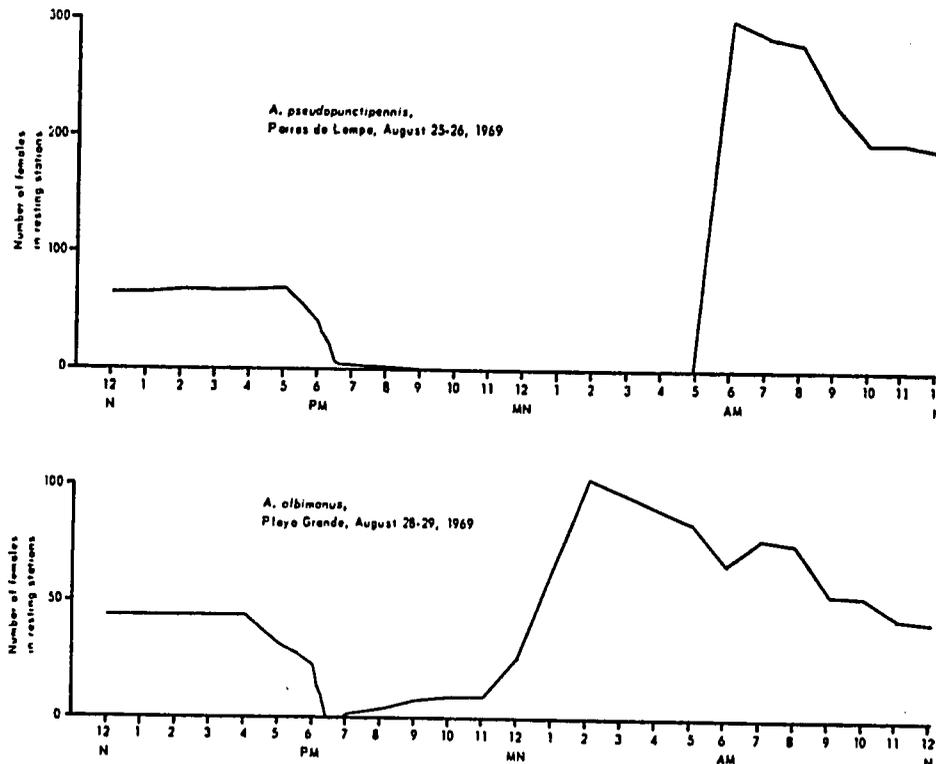


FIG. 2.—Diel resting cycle of *Anopheles albimanus* (below) and *A. pseudopunctipennis* (above) females in El Salvador, August 25-29, 1969.

there was mass movement into the daytime resting places with peak numbers being found between 6:00 am and 8:00 am. Between 8:00 am and 10:00 am there was some "resettling," but numbers were stable between 10:00 am and 5:00 pm.

The reciprocal of the resting curve represents the activity patterns of the two species. Thus, it is seen that *A. albimanus* females depart daytime resting places at dusk and are most active between dusk and midnight. These are the hours when maximum activity would be expected regarding its flight and feeding habits. On the other hand, *A. pseudopunctipennis* females, and males of both species, have an activity pattern lasting throughout the hours of darkness.

These observations have important implications. For example, aerial ULV treatments are normally conducted at a time of the day (5-6 am) when anophelines are in protected niches of daytime resting places, whereas the first several hours after dark should be optimum from the standpoint of exposed mosquitoes. Also, we know from current host preference studies that a high percentage of blooded females of *A. albimanus* found in houses during the early morning hours have fed on something other than man. This implies that a portion of blood-fed specimens enters houses solely for shelter and not for blood. Therefore, it seems possible that the commonly used terms endophilic and endophagic to describe anopheline preference for resting and feeding indoors, respectively, might represent distinct entities for *A. albimanus*. Perhaps the truly endophagic portion of the population is that portion which enters a dwelling during the early evening hours seeking blood while the shelter-seeking females already having had a blood meal are not endophagic at all, and are endophilic only by the chance of being nearer to a dwelling than a tree cavity or some other natural or artificial shelter. This aspect

is being pursued by studying blood meal sources of *A. albimanus* collected from dwellings during various periods of the night. Other studies are planned to determine the effect of ovarian development on the resting activity cycle of *A. albimanus*.

SUMMARY

Research objectives of the Central America Malaria Research Station (CAMRS) in El Salvador require means of measuring anophelism apart from commonly used domicile methods. *Anopheles albimanus* and *A. pseudopunctipennis* are present in large numbers in natural diurnal resting sites, and are less abundant in artificial sites. Favored natural sites are "rock-oriented" and include crevices of large and small rocks, rock walls, and lava deposits in shaded areas. Tree trunks, cavities, and roots also offer favorable sites. From natural sites, 181 collections yielded 7,670 *A. albimanus* and 2,344 *A. pseudopunctipennis* for an average of 42.4 and 12.0, respectively. Of these, the sex ratio of *A. albimanus* was 24.5 percent males and 75.5 percent females, while that of *A. pseudopunctipennis* was 34.0 percent males and 66.0 percent females. *A. pseudopunctipennis* seemed to prefer darker, more protected niches than *A. albimanus*. *A. albimanus* females moved into resting sites at dawn, departed at dusk, and returned between midnight and 3 am, whereas *A. pseudopunctipennis* females were absent from resting sites throughout the period of darkness. The results emphasize the usefulness of diurnal resting sites for density measurements associated with control projects and biological studies, and as a source of specimens for experimental use.

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