THE HOUSE-FREQUENTING, HOST-SEEKING AND RESTING BEHAVIOR OF *ANOPHELES DARLINGI* IN SOUTHEASTERN AMAZONAS, BRAZIL[1]

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ABSTRACT. Studies on the behavior of *Anopheles darlingi* were conducted at a site along the Ituxi River, Amazonas, Brazil. Patterns of host-seeking activity inside and outside a single-walled house both presented activity peaks at sunset and sunrise, but biting activity inside a four-walled house peaked after sunset then gradually decreased during the night. Major movements of females into and out of the four-walled house occurred at sunset and sunrise, respectively. Marked engorged and unengorged females released indoors were observed to preferentially rest on the ceiling. These behavior patterns were confirmed by replication and/or with more than one sampling technique or study method. Additional observations on exit sites, spatial distribution of resting females and physiological condition of exiting specimens were recorded.

INTRODUCTION

Anopheles darlingi Root is the primary vector of malaria in the Brazilian Amazon Basin and is equally important in many other areas of its discontinuous range from southern Mexico to northern Argentina (Giglioli 1956, Root 1926, Davis 1931, Forattini 1962). Consistent with the behavioral characteristics of other important malaria vectors, *An. darlingi* is thought to be endophagic and anthropophilic. It is generally characterized as having a peak of indoor biting activity around midnight, although different patterns of biting activity have been documented (Deane et al. 1948, Charlwood and Hayes 1978). In addition, *Anopheles darlingi* is described as endophilic, i.e., feeds and rests indoors; however, partially exophilic and/or zoophilic behavior also has been reported (Rachou 1958).

Our understanding of the behavior and ecology of *An. darlingi* in the Amazon Basin, as it relates to malaria control derives mainly from the research of the 1940s and 1950s. More current information is required, from almost all regions, to properly assess the effectiveness of accepted malaria control operations. Included in this report are observations on the behavior of host-seeking populations of *An. darlingi* along the Ituxi River in Amazonas, Brazil.

MATERIALS AND METHODS

Study site. The Ituxi River is a tributary of the larger Purus River (Fig. 1) with headwaters in Acre and Amazonas states. The Ituxi-Purus confluence is located approximately 10 km west of Labrea in Amazonas State. The "terra firme" and "igapo" habitats are the most frequently encountered habitats along the river (terra firme refers to highland that is not normally inundated by the river while igapo is composed of low areas that are inundated for several months each year).

Residents generally spend their entire lives on this river system and comprise a widely distributed but stable community. Houses normally are built on terra firme near the edge of the river. The economy is based on hunting, fishing and subsistence farming, as well as collecting rocks for construction and harvesting rubber (from *Hevea brasiliensis*), latex (from *Achras* spp.) and Brazil nuts. Houses are constructed on stilts with palm thatch roofs, and often have only one or two walls (some have none) which are made of palm slats, as are the floors. In spite of active control efforts the Ituxi River population is characterized by a persistently high prevalence (7.8%) of malaria, with 47.8% due to *Plasmodium falciparum* and 52.2% due to *P. vivax* (Alecrim 1979)[5].

Based on data from a survey of mosquito populations along the Ituxi River in July–

[1] This research was conducted at the Nucleo de Medicina Tropicál e Nutricão, Universidade de Brasilia, Brasilia, D.F., Brazil, and was supported by the Brazilian Conselho Naçional de Pesquisas and Research Contract No. DAMD 17-79-G-9450 from the U.S. Army Medical Research and Development Command, Office of the Surgeon General, Ft. Detrick, Frederick, MD 21701. The opinions contained herein are those of the authors and should not be construed as official or reflecting the views of the Department of the Army.

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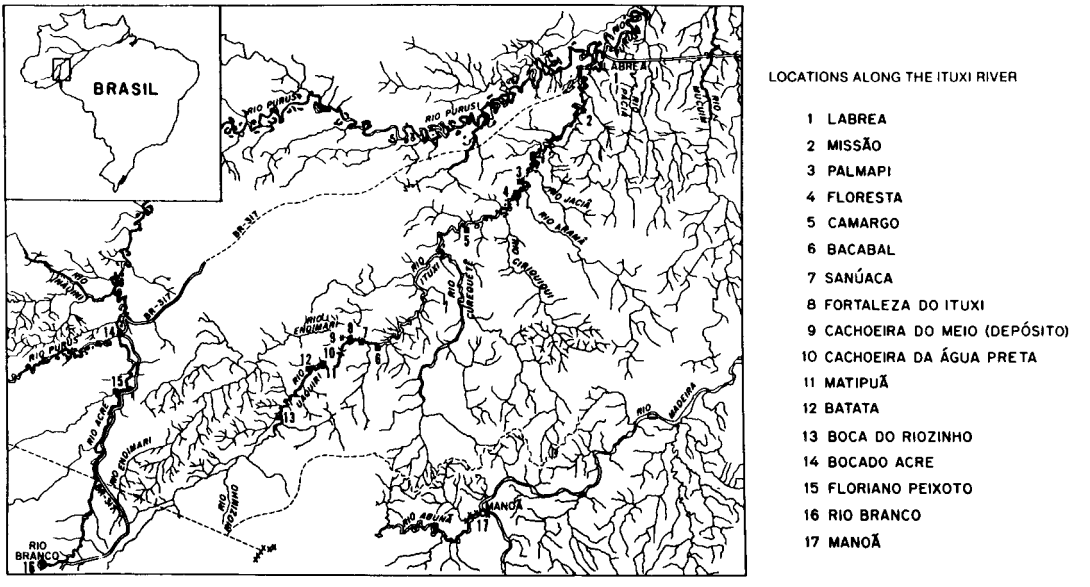

Fig. 1. Map of the Ituxi River drainage system with location of the study site at Floresta (4).

August 1978, we selected Floresta as our study site. In January 1979 an experimental house was built there on stilts 85 cm above ground, with a palm thatch roof, walls of palm slats, one small room with a wood plank floor and another with a floor of palm slats. The experimental house was located within 100 m of the two families that lived at Floresta. A ladder and rafter system were constructed in the house to facilitate access to the ceiling for studies of preferred resting sites. Large openings (greater than 5 cm) between rooms and between the roof and outer walls were screened to reduce mosquito movement.

Window traps were placed in the window spaces. Openings to accommodate two traps later were constructed in one gable of the house. The lower ledges of windows and window traps were 2 m above the ground, whereas traps located in the gable were 3.7 m above ground. In addition, two modified veranda traps were constructed for sampling populations moving through openings in the walls and corner of the house. One trap enclosed a relatively large external wall surface (1.8 x 2.95 m) which included an exit trap in one window, whereas the second veranda trap enclosed only the corner section (1.8 x 0.2 x 0.2 x 1.8 m) at the juncture of two walls.

Survey. A survey for *Anopheles darlingi* populations, employing human bait collections, was conducted along the Ituxi River in July and August 1978.

Biting activity. An initial series of studies on vector behavior was conducted in August 1978 at Floresta in a house with only one wall, that had been sprayed with DDT in April 1978. This consisted of a sequence of three all night human bait collections that were conducted for 30 min each hour with one team of collectors (2 men per team) in the house and another team in an open area about 20 m from the house.

Another three series of studies on activity patterns were conducted in the experimental house. Uniform methods were employed; thus, findings are presented as combined results with reference to the separate series as study 1 (February–March 1979), study 2 (May–June 1979) and study 3 (March 1980). Weather conditions were similar for studies 1 and 3 with temperature ranging from 24 to 31°C compared with 16–30°C for study 2. Paired, outdoor-indoor human bait collections (1 collector/site) were conducted in a uniform manner throughout with collection intervals of 10–15 min/hr. The 10 min collection interval was employed for only one series because of very dense anopheline populations. Collectors were continually rotated between collecting sites and teams were switched every 6 hours. Furthermore, teams were rotated between shifts every night. Temperature and humidity were recorded every 6 hours for study 1 and at hourly intervals for studies 2 and 3.

House entry/exit times and locations. Entrance and exit traps were emptied at 2 hr intervals. In March 1980, an entrance and an exit trap each were placed in the gable of the house, veranda trap collections also were conducted in March 1980.

Sella's method for evaluating the stages of

blood meal digestion and ovarian development was employed with specimens caught exiting the house. The effectiveness of Sella's method for assessing the physiological condition of *An. darlingi* specimens was reported separately (Roberts et al. 1983). In the interest of brevity and simplicity, unengorged specimens captured in exit traps were excluded from analyses presented in this paper.

Resting sites. Blood engorged *An. darlingi* were collected in the peridomiciliary environment during the early evening, marked with USR fluorescent pigment 1953 and released inside the house at 2200 hr. Periodic observations with a Blak-Ray®, ULV.56, long wave ultra-violet lamp were made following release to determine the preferred resting site of blood-fed specimens. These tests were conducted on two separate occasions during study 1 and 100 specimens were marked for each test.

On two occasions specimens were collected from the entrance traps between 1800 and 2000 hr, marked and then released within the house at 2040 hr. Periodic observations with the ultra-violet lamp were made following release to identify resting sites of non-engorged females. All specimens caught during each release night were inspected for the presence of marked specimens.

Series of resting collections were conducted for 5 min simultaneously inside the house, from the external walls and from vegetation near the house for two nights during study 1. A series of the 5 min collections was conducted at half hour intervals from 1830 to 2105 hr and another from 1740 to 1915 hr. The resting adults were captured with a mechanical aspirator.

A single collector was stationed at each of five sites; one less than 10 m from the house, another at 20 m and the third at 40 m from the house. Continuous 15 min captures were conducted from 1750 to 2005 hr on June 3 and 4, 1979.

Statistical analyses. To test the effect of low ambient temperatures on host-seeking activity we analyzed collection data for two activity intervals, 1835–2055 hr and 2345–0500 hr, from studies 1 and 2 with the Kendall Rank Correlation and Kendall Partial Rank Correlation Coefficients (Siegel 1956). Data consisted of numbers collected per collection, time of collection and temperature at the time of each collection.

Separate Kendall Rank Correlation Coefficients were calculated for numbers vs. temperature, number vs. time and temperature vs. time for both activity intervals. Tests of significance were performed on their values at the 0.01 level of probability. The r values were then employed in the Kendall Partial Rank Correlation Coefficients to parcel out the time and time-temperature effects. No tests of significance were available for the resultant values.

RESULTS

Survey. Populations of *An. darlingi* were consistently present in the peridomiciliary (within 10 m of the house) habitats sampled (ca. 15) along the Ituxi River system. Only at Fortaleza, an isolated site with cattle, were anopheline species other than *An. darlingi*, vis. *An. oswaldoi* (Peryassu) and *An. nuneztovari* Gabaldon, encountered in abundance in the cleared areas near the houses.

Biting activity. Peak biting activity of *An. darlingi* "indoors" and outside of a house with only one wall occurred at sunset and sunrise (Fig. 2). Although the house had been sprayed with DDT, no marked differences were found in the "indoor"/outdoor activity patterns.

A bimodal pattern of biting activity outdoors, as reported above, was duplicated in studies 1 and 3 with collections conducted near the experimental house. The peak at sunrise was not apparent in data from study 2. In addition, we collected females coming to human bait during daytime outdoors during study 2. There was an absence of activity only in the early afternoon.

Study 1 collections revealed a sharp increase in indoor biting after sunset and persistent but declining activity throughout the night (Fig. 3). The minimum temperature recorded during

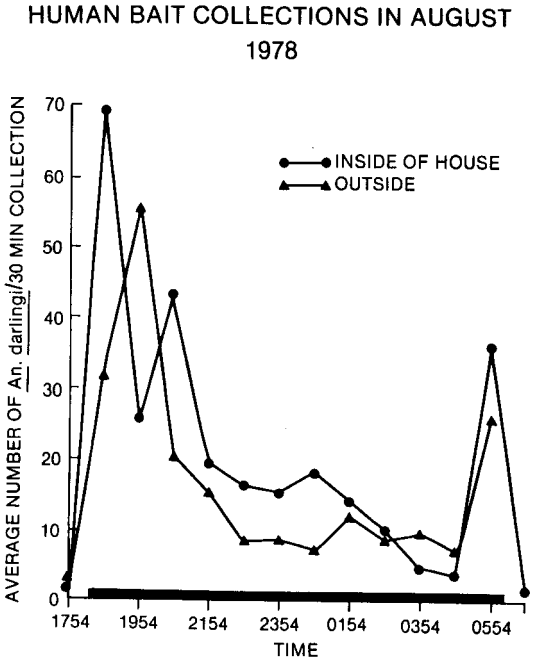

Fig. 2. Number of *Anopheles darlingi* from 3 nights of human bait collections at Floresta, Ituxi River, Amazonas, Brazil in August 1978. Data plotted by mid-time of the 30 min collection intervals. Collections conducted by 2 collectors for 30 min each hour.

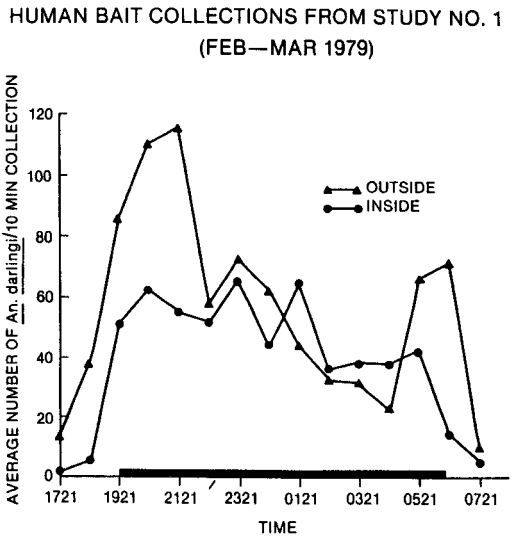

Fig. 3. Number of *Anopheles darlingi* from 3 nights of human bait collections at Floresta, Ituxi River, Amazonas, Brazil in Feb–Mar, 1979 (study 1). Paired indoor/outdoor collections were conducted for 10 min each hour and data are plotted by mid-point of the collection intervals.

these collections was 24°C. There were no significant differences, as determined by the Kolmogorov-Smirnov two sample test, in activity patterns between studies 1 and 3. In contrast, biting activity in study 2 dropped after 2147 hr and remained low the rest of the night (Figs. 3 and 4). The Kolmogorov-Smirnov two sample test revealed significant differences ($P < 0.01$) between activity patterns of studies 1 and 2.

We hypothesized that the low late-evening temperatures during study 2 suppressed host-seeking activity (Fig. 4). The low temperatures (16°C) experienced during study 2 typifies a "friagem" phenomenon resulting from a large cold front moving north-northeast from the Andes into the western Amazon Basin (IBGE 1977). The Kendall Rank Correlation and Kendall Partial Rank Correlation Coefficient analyses of data from two activity intervals for both studies gave highest rho values for numbers collected vs. temperature, r values of 0.7 ($P > 0.01$) and 0.64 ($P < 0.01$) respectively. When contributions of time on numbers collected were parcelled out with the Kendall Partial Rank Correlation Coefficients, the r values for numbers collected vs. temperature remained high, 0.7 and 0.55 respectively.

House entry/exit times and locations. A surge of mosquitoes entered the house from 1800 to 2200 hr (Figs. 5 and 6C). Only collections during study 3 showed an increased ingress at sunrise (0430–0630 hr) and over 50% of the total was obtained from a single collection during and following an episode of heavy rain and high winds.

Exodus from the house began after 0400 hr. Again, there were marked differences in results for study 2. The ingress of females peaked earlier (1800–2000 hr) and was of short duration in study 2; in addition, movement out of the house started later (0600–0800 hr) and continued through mid- to late-morning. A majority of exiting females captured after 0400 hr in all studies were late fed or Sella Stage 2 or greater.

Actual numbers entering via the gable were consistently less than entering through the windows (Figs. 6A and 7) and there was no early evening peak of ingress. Again, the surge of mosquitoes entering the house via the windows during the 1630–1830 hr interval of study 3 was produced by a single, unusually large collection.

The physiological condition of engorged *An. darlingi* collected at different exit portals in the house was extremely variable. Most specimens that exited through the wall during early evening were recent fed, as represented by collections from veranda trap A (Fig. 6B), while those captured at sunrise were predominantly late fed. Similar findings were obtained with females that exited at the corners of the house (Fig. 6D). Proportionally more specimens captured escap-

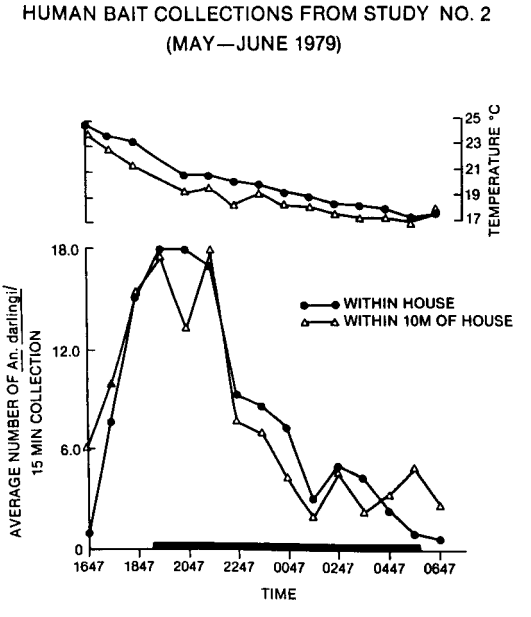

Fig. 4. Numbers of *Anopheles darlingi* from 3 nights of human bait collections at Floresta, Ituxi River, Amazonas, Brazil during study 2 (May–June, 1979). Data from paired indoor/outdoor 15 min collections conducted each hour during the night and plotted by mid-point of the collection intervals.

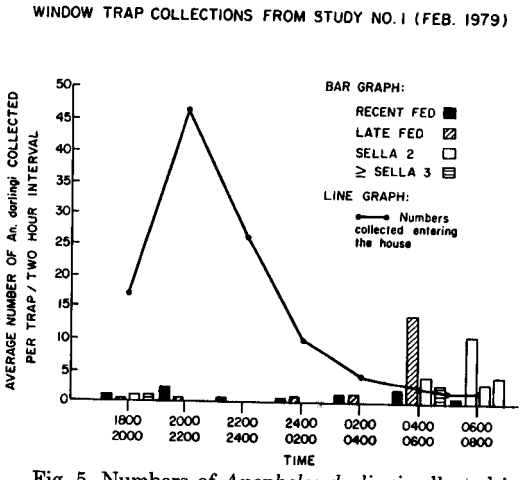

Fig. 5. Numbers of *Anopheles darlingi* collected in entrance and exit traps located in an experimental house at Floresta, Ituxi River, Amazonas, Brazil during study 1 (Feb–Mar, 1979). Collections were conducted in 2 hr intervals throughout the night for 3 nights. Numbers of engorged/gravid females captured exiting the house are plotted by physiological condition at time of capture.

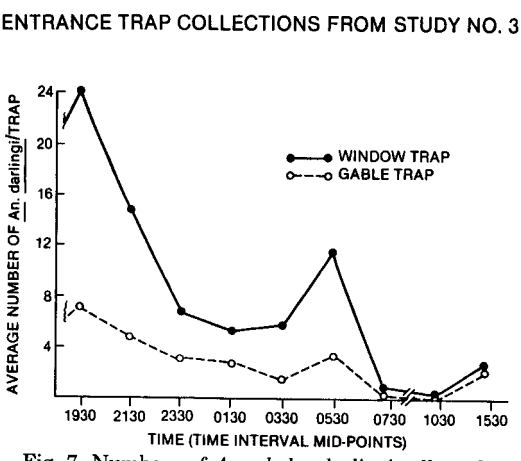

Fig. 7. Numbers of *Anopheles darlingi* collected in entrance traps placed in windows and in the gable of an experimental house at Floresta, Ituxi River, Amazonas, Brazil during March, 1979. Collections were conducted in 2 hr intervals throughout the night for 3 nights. Data plotted by mid-point of the 2 hr collection intervals.

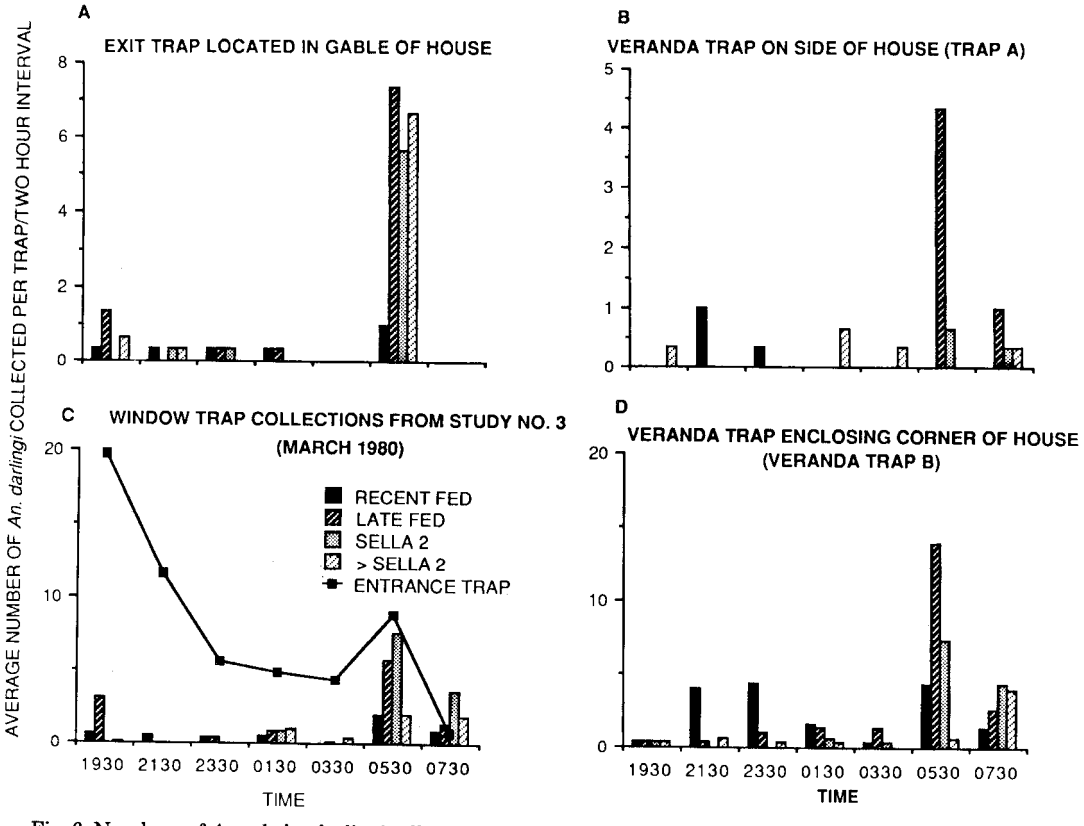

Fig. 6. Numbers of *Anopheles darlingi* collected in entrance, exit and veranda traps located in an experimental house at Floresta, Ituxi River, Amazonas, Brazil during study 3 (March, 1980). Collections were conducted in 2 hr intervals throughout the night for 3 nights. Numbers of engorged/gravid females captured exiting the house are plotted by midpoint of the 2 hr intervals and by physiological condition at time of capture.

ing directly through the windows were late fed and Sella Stage 2, with Sella Stage 2 females being most abundant at sunrise (Fig. 6C). Recent fed females were relatively uncommon in collections from the gable of the house (Fig. 6A). Although overall numbers of specimens captured in this trap were low, the collections at sunrise were large, with a strong representation of Sella 3+ females.

We compared preferences for exit sites by calculating ratios of numbers of exiting females by surface area of wall sampled. An 89-fold greater number escaped through a given area of window (0.25 m^2) as escaped via cracks in the wall (5.31 m^2), average per trap of 41 and 9.6 specimens respectively. Only twice as many (0.2475 m^2) escaped via the window as escaped through an equivalent area at the corner of the house (veranda trap B). However, for Sella Stage 3 and greater females, more than twice as many escaped via windows than through the corner of the house. More females escaped through the window space than through an equal area in the gable of the house, however, for females of Sella Stage 3 and greater, a greater number exited via the gable than through windows.

Resting sites. The number of engorged *An. darlingi* that were observed inside the house progressively declined with time after release. These results were consistent in both mark-release studies. Less than 50% could be located within the house at 1 hr postrelease and only 20% were still in the house 7–8 hr postrelease. Of those resting indoors, 58.7%, 37% and 4.3% were observed resting on the ceiling, walls and floor, respectively. Very few marked specimens were found outside the house prior to sunrise and there was no apparent preference for outside resting sites.

Marked unengorged specimens also more commonly rested on the ceiling (Table 1). However, there was a more equal distribution of numbers resting on the walls and ceiling later in the night. After chronological adjustments in numbers of marked specimens following removal by trapping, we found a cumulative 32.9% of marked specimens were captured in human bait collections and 9.5% in exit trap collections (Table 2). Most (59%) marked individuals collected from human bait fed during the first 3 hr after release. Only 2 of 11 marked specimens recaptured in exit traps were collected before sunrise.

Collections of resting *An. darlingi* were conducted inside and outside the house. Five females collected inside prior to 1935 hr were unfed, but all subsequent specimens (total of 8) were engorged. Most specimens caught outside during the evening were unfed (55/62). However, 3 of 5 specimens collected from 0540 to 0715 hr were engorged.

Table 1. Number of marked* female *Anopheles darlingi* observed by resting site at intervals throughout the night in an experimental house located at Floresta on the Ituxi River, Amazonas, Brazil. A total of 81 and 82 marked, unfed specimens were released June 1 and 2, 1979, respectively.

Hours after release	Number resting by site within the house				
	Floor	Walls	Rafters	Ceiling	Totals
0.5[a]	5	14	7	28	54
1.0[b]	0	10	4	27	41
1.5[a]	0	10	3	25	38
$\bar{x}$	1.7	11.3	4.7	26.7	44.3
4.5[a]	0	14	0	13	27
5.0[b]	0	10	1	7	18
$\bar{x}$	0	12	0.5	10	22.5
8.5[a]	0	7	0	11	18
8.5[b]	0	7	0	6	13
$\bar{x}$	0	7	0	8.5	15.5

* Females were marked with USR pigment 1953 and marked specimens were identified with a Blak-Ray, ULV; 56, long wave ultra-violet lamp.

[a] Observations made on the 81 specimens released June 1, 1979.

[b] Observations made on the 82 specimens released June 2, 1979.

Spatial distribution of host-seeking females. Prior to sunset more specimens of *An. darlingi* were collected in the forest, but during and after sunset most specimens were collected near the house (Table 3). Only specimens of *An. darlingi* were collected near the house, whereas we observed considerable species diversity at sites 20 (with 8 species) and 40 m (with 9 species) from the house (Table 4).

DISCUSSION

During this investigation we documented several distinct behavioral characteristics of *An. darlingi* populations within and near human habitations. The pattern of host-seeking activity in a house with a single wall was essentially identical to the pattern of activity outside. The four definable intervals of outdoor biting activity consisted of low activity during the day, peak activity for about 3 hr during and after sunset, moderate to low activity during the 2200–0530 hr interval and a secondary peak of intense activity at sunrise. These patterns are in general agreement with those reported for *An. darlingi* populations reported by Charlwood and Hayes (1978) from collections conducted on a veranda of a house in Aripuana, Mato Grosso, Brazil.

Biting activity within the experimental house

Table 2. Number of marked specimens available, number collected, percent collected and cumulative percentage of marked* female *Anopheles darlingi* recaptured in human bait and exit trap collections in an experimental house at Floresta, Ituxi River, Amazonas, Brazil. Combined data from releases of 81 and 82 marked, unfed specimens at 2040 hr on June 1 and 2, 1979, respectively.

Time	Human bait				Exit trap			
	Number[a] available	Number collected	Percent collected	Cumulative percentage	Number[a] available	Number collected	Percent collected	Cumulative percentage
2000–2200	163	16	9.8	9.8	147	2	0.14	1.4
2200–2400	145	15	10.3	20.1	130	0	0	1.4
2400–0200	130	8	6.2	26.3	122	0	0	1.4
0200–0400	122	5	4.1	30.4	117	0	0	1.4
0400–0600	117	3	2.6	32.9	114	0	0	1.4
0600–0800	113	0	0	32.9	113	6	5.3	6.7
0800–1200	106	—	—	—	107	3	2.8	9.5
1200–1800	103	—	—	—	103	0	0	9.5

* Females were marked with USR pigment 1953 and marked specimens were identified with Black-Ray, ULV;56, long wave ultra-violet lamp.

[a] Number of marked specimens available for recapture.

Table 3. Number of *Anopheles darlingi* in human bait collections at 3 sites near an experimental house at Floresta, Ituxi River, Amazonas, Brazil (June 3 and 4, 1979).

Collection time	10 m from house			20 m from house*			40 m from house*		
	June 3	June 4	$\bar{x}$	June 3	June 4	$\bar{x}$	June 3	June 4	$\bar{x}$
1750–1805	6	5	5.5	2	7	4.5	10	10	10
1835–1850	40	33	36.5	12	6	9.0	0	10	5
1900–1915	127	58	92.5	4	15	9.5	10	6	8
1925–1940	44	21	47.5	1	23	12.0	15	2	8.5
1950–2005	87	20	53.5	2	21	11.5	0	7	3.5

* Collection site located in a low secondary forest.

Table 4. Species and numbers of specimens captured in human bait collections conducted simultaneously at 3 sites (10 collections/site) near an experimental house at Floresta, Ituxi River, Amazonas, Brazil. Collections were conducted for 15 minutes each from 1740–2005, June 3 and 4, 1979.

Distance from experimental house		
10 m	20 m	40 m
Anopheles	*Anopheles*	*Anopheles*
darlingi (539)	*darlingi* (106)	*darlingi* (78)
	nuneztovari (21)	*mediopunctatus* (7)
	oswaldoi (19)	*nuneztovari* (4)
	mediopunctatus (19)	*oswaldoi* (3)
	peryassu (2)	*peryassu* (1)
	shannoni (1)	*numbus* (1)
	Aedes	*Aedes*
	fulvus (2)	*fulvus* (2)
	Psorophora	*Culex*
	cingulata (1)	*spissipes* (1)
		Psorophora
		cingulata (3)

with complete walls increased after sunset and gradually decreased during the night. Unfed females moved into the house during and immediately after sunset and at sunrise engorged/gravid females moved out of the house. Although the bimodal biting pattern outdoors was not duplicated indoors, we propose that movement into and out of the house at sunset and sunrise, respectively, was partial expression of these peaks in activity.

The activity patterns were reasonably consistent throughout our investigations, even

though the amplitudes and duration of peak activities and relative levels of activity under conditions of low population densities varied considerably. In fact, with subsequent studies under conditions of low population densities we found patterns of activity indoors that were similar to the patterns of activity outside.

The effects of population density on activity patterns of malaria vectors have been discussed by Elliott (1972). In our studies low population densities seemed to result in shorter periods of peak activity and disproportionately lower levels of activity during non-peak periods. These differences in activity patterns are probably an affect of sample bias as influenced by vector behavior and sensitivity of the human bait collection to population densities. First, it seems likely that individual mosquitoes that do not successfully take a blood meal during peak periods of activity will continue, at reduced levels of activity, to seek a host as long as environmental conditions are favorable, i.e., there is an overflow of activity into non-peak activity periods. This overflow will substantially affect the pattern of biting activity if a greater proportion at high population densities do not succeed in feeding. Second, the sample method, under specified environmental and meteorological conditions, has a definable sensitivity to the absolute density and level of activity of the population sampled. There is probably a sliding scale relationship between sample sensitivity and a given set of environmental conditions, absolute population density and level of activity according to time of day or night. Thus, we can reasonably expect variable patterns of activity under variable conditions in the field. Furthermore, the low ambient temperatures during study 2 resulted in a suppression of biting activity at night and was illustrative of vector sensitivity to environmental conditions.

Our observations on the patterns of indoor biting activity were verified by the mark and release of unfed females. Most marked specimens obtained in human bait collections were recaptured during the first 3 hours after release. The recapture of marked females in exit traps also verified our finding that most engorged females collected in exit traps were captured during the sunrise interval and, of these, most had fed several hours prior to capture. In addition, the observation that marked females congregated in corners of the house gave us the first clue that this was a major exit portal, which was later confirmed with veranda trap collections. We also noted that marked females seemed to move down the ceiling and congregate near the eaves just prior to sunrise, but we have no exit trap data to support this observation.

Marked females disappeared more rapidly from the experimental house after release than we expected from exit trap data with non-marked females. Furthermore, since there was no surge in numbers of marked females in exit traps at the time of release, we believe these specimens fled immediately through cracks in the floor (cages were placed on the floor at time of release) and walls. Agitation associated with the marking procedures may have stimulated most females to escape, however, marked females that remained within the house seemed to behave similarly to unmarked (undisturbed) populations.

Deane and Damasceno (1948) reported that *An. darlingi* in Belem, Brazil preferred to rest on the lower meter of the house wall. However, the engorged and unengorged females that we marked and released within the house rested primarily on the ceiling. An exit trap placed in the gable of the house provided additional verification of this observation. Relatively large numbers of late fed, Sella Stage 2 and Sella Stage 3 (or greater) females were captured in this trap, which seems to indicate that many of the females were resting in the palm thatch roof for variable but lengthy periods of time after taking a blood meal. This preference for resting on the ceiling could simply reflect regional differences in *An. darlingi* populations. Similar studies on indoor resting sites should be conducted in other geographical areas, as this has significant implications for malaria control spray policy. Obviously, the risk of the spray settling back onto the individual applying spray to the ceiling is a serious consideration. However, our observations on the behavioral response of *An. darlingi* to DDT (unpublished data) suggest that spraying of housewalls and the lower part of the ceiling, as currently performed, is adequate to control malaria transmission.

We conclude from these studies that *An. darlingi* populations at the Ituxi River study site were endophagic, anthropophilic and only partially endophilic (rest indoors through the gonotrophic cycle). However, the evidence is strong that behavioral differences do exist from one area to another, e.g., during preliminary studies in the state of Para the senior author observed peak biting activity of *An. darlingi* to occur after midnight. Hudson (1984) has recently reported that *An. darlingi* in Suriname did not have a secondary peak in biting activity at sunrise. This peak in activity is similarly absent in *An. darlingi* populations north of Manaus (Charlwood and Hayes 1978). Such differences raise the possibility that *An. darlingi* is a species complex, a subject that warrants coordinated research efforts throughout the geographical range of this important malaria vector.

ACKNOWLEDGMENTS

The authors express their sincere appreciation to Professor Aluzio R. Pratá, Director of the Nucleo de Medicina Tropical e Nutriçâo, Universidade de Brasilia, Brazil and Dr. Heitor Dourado, Director, Instituto de Medicina Tropical de Manaús, Manaús, Brazil for their guidance and support in this research effort. Technical assistance provided by Srs. Jose Bento Lima and João Dalmacio also is gratefully acknowledged.

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