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ECOLOGIC OBSERVATIONS ON ANOPHELINE VECTORS OF MALARIA IN THE BRAZILIAN AMAZON

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Abstract. Human intervention in the Brazilian Amazon region promotes contacts between humans and vectors that may favor the propagation of anopheline mosquitoes and the spread of malaria in the absence of planning and infrastructure to control this disease. Vector ecology studies were carried out to determine the risk areas. These data should help in designing appropriate malaria control measures. Data from 14 different regions are reported. Vectors are able to adapt to different environments, which made it necessary to study each area. The parameters studied were *Anopheles* breeding sites, species distribution, incidence, feeding preferences, hours of maximum activity of adult mosquitoes, seasonality, resting places, and the presence of *Plasmodium.* Species complexes were also studied. *Anopheles darlingi* may be responsible for maintaining malaria in human populations in this region. A reduction in the population density of *A. darlingi* in a particular geographic area can sometimes cause the disappearance of malaria. This species feeds at night but has a peak of activity at the beginning of the evening and another at dawn. Other species are mainly crepuscular and all anophelines demonstrated pronounced exophilia. The timing of feeding activities was found to vary in areas altered by human intervention and also depended on the time of the year and climatic conditions. The larvae were more abundant in the rivers with a less acidic pH and rural areas showed the highest larval index.

Natural ecosystems throughout the world are being severely altered by human intervention. Population pressures bring about the construction of hydroelectric dams, irrigation projects, open pit mines, and uncontrolled human colonization. Such activities often favor the propagation of anopheline mosquitoes and the spread of malaria. This has been shown in several countries especially in regard to irrigation canals and the lakes formed by hydroelectric dams.[1–3]

As a result of new human activities in the Brazilian Amazon region, cases of malaria have been increasing in recent years (Figure 1). Some of the environmental changes now occurring in northern Brazil are the construction of hydroelectric dams, roads, and pipelines. There are also extensive mining and lumbering operations and rural settlements. Malaria has emerged in some areas and re-emerged in others, pressured by changes in human activity.[4, 5] Contributing factors are the presence of the anopheline vectors and a lack of planning and infrastructure to control this disease.[6]

Vector ecology studies were done to determine the risk areas for malaria and to formulate appropriate malaria control measures. Fourteen different regions were studied, including five in Rondonia State in southern Amazonia, two in Amazonas State in central Amazonia, one in Roraima State in northern Amazonia , one in Amapá State in northeastern Amazonia, and two in Para State on the eastern coast. The parameters studied were *Anopheles* breeding sites, species distribution, incidence, feeding preferences hours of maximum activity of adult mosquitoes, seasonality, resting places, and the presence of *Plasmodium vivax* and/or *P. falciparum.* Species complexes were also studied.

MATERIALS AND METHODS

Study sites. Larval and adult mosquitoes were captured in urban and rural areas, at road sides, near new immigrant dwellings, at open mines (garimpos), by rivers in ecologic reservations, near hydroelectric dam sites, and along an oil and gas pipelines (Figure 2). Mosquito collections were obtained mainly between 6:00 PM and 10:00 PM, except when hourly collections were taken until dawn. The state, collection dates, number of areas selected, and the total number of sites searched in each locality are listed in Table 1. The Manaus and Urucu-Coari programs are still going on.

In 1993, surveillance of Manaus (locality no. 2) included the entire city. Once the problem areas were identified, the collections were concentrated at the periphery, where uncontrolled human invasions were occurring. Localities no. 4 (University of Porto Velho), 7 (Maracá), 9 (Samuel), 10 (Balbina), and 11 (Porteira) had two different areas surveyed in each. One was a natural ecosystem and the other had been altered by human interventions. The Urucu-Coari study (localities 13 and 14) also included two areas: a camp, which had a well-organized mosquito control program and the edges of the Urucu River, where occasional small farms were located. At some of the hydroelectric dams, surveillance was carried out before, during, and after impoundment of the artificial lake.

Anopheline collections. *Larvae.* For collecting larvae, an area of 3 m^2 was selected at the margin of a breeding site. A white enamel tray was used to dip out (collect) third and fourth instar larvae and pupae. These insect stages were then transferred with a medicine dropper to a transport jar. The tray was immersed several times until no more larvae could be seen. In smaller breeding areas, the dipping procedure was the same. Most larvae were fixed with McGregor's solution and sent to the laboratory for identification. Potassium hydroxide solution or creosote were used for clearing the specimens. Some larvae were taken back to the laboratory alive so that they could be reared to the adult stage and then identified.

At the Balbina hydroelectric dam, the following physicochemical factors were measured: temperature (using a thermometer submerged to a depth of 20 cm), oxygen chemical demand (by titration with potassium permanganate), pH (by means of a portable pH meter); and inorganic nutrients (Ca^{++}, Mg^{++}, and dissolved Fe). The species of algae present

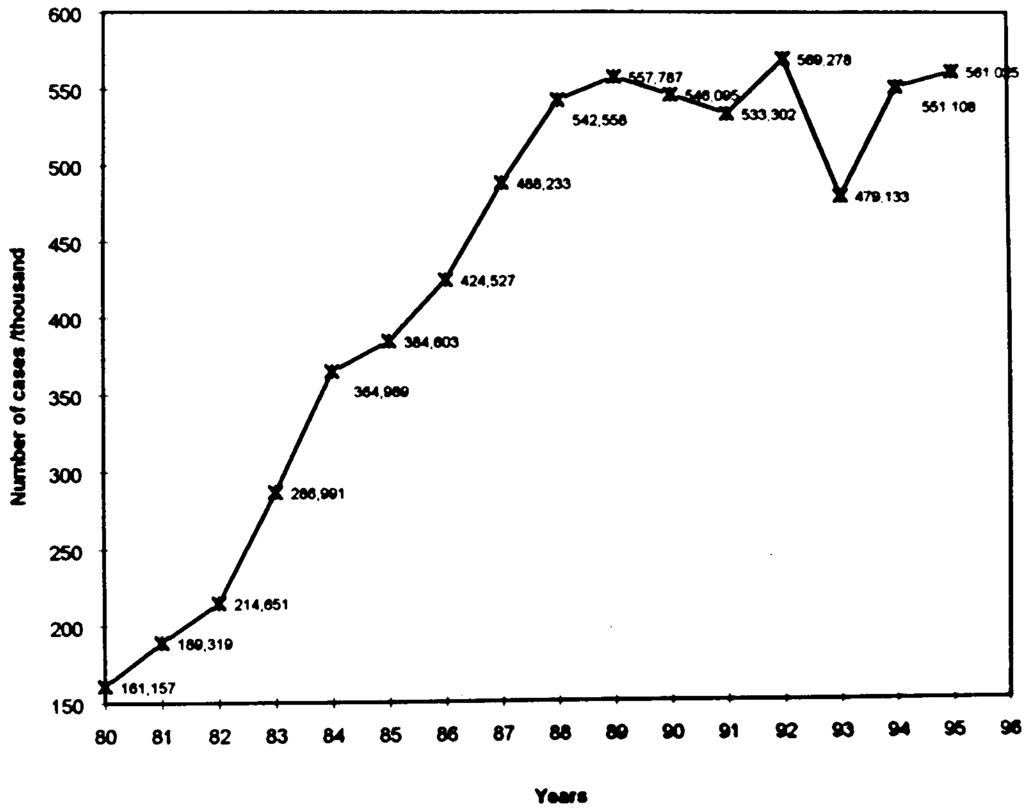

FIGURE 1. Malaria in Amazonia, Brazil, 1980–1986 (source: National Health Foundation, Brasilia, Brazil).

were also identified to determine the conditions of the breeding sites.

Adults. **C**ollectors normally worked alone at one site over a 4-hr period with resting periods of 5 min after each hour. They sat inside houses (intradomicilliary collections), 1–5 m from a house (peridomiciliary collections), more than 5 m from a house (extradomicilliary collections), at the edge of or within the woods. The anophelines were collected with an aspirator when attempting to blood-feed, transferred to plastic jars, and labeled.

Identification. The Gorham, Stojanovich, and Scott[7] illustrated keys were used as an aid in the identification of the mosquitoes. When necessary, the eggs, fourth instar larvae, or the male genitalia were examined.

Identification of *Plasmodium* spp. in infected mosquitoes. Mosquitoes were triturated individually on microplates containing boiled casein solution containing NP40. The triturates were tested with specific monoclonal antibodies and conjugates for *P. falciparum* and *P. vivax*[8, 9] supplied by Dr. Robert Wirtz (Walter Reed Army Institute of Research, Washington, DC). To study the karyotypes of anopheline populations, the metaphasic stages of cerebral ganglion cells were stained with Giemsa and silver nitrate.

For electrophoretic analysis, descendants of females captured in the wild were used. An average of 2–4 individuals were used from each group. Electrophoresis with approximately 25 enzymes in the different *Anopheles* populations was carried out in horizontal starch gel, starch agarose, and acrylamide.[10, 11]

RESULTS

In the present study, we identified 26 anopheline species in collections from 14 areas. Some adults are not anthropophilic; therefore, only immature stages could be collected. The collecting sites were in the northern, southern, southwestern, central, and eastern regions of the Brazilian Amazon. The species found in natural and altered environments (larvae and/or adults) are listed in Table 2. *Anopheles nuneztovari* was the only species found in all 14 areas while *An. squamifemur* was encountered in only one locality and *An. neivai* and *An. nimbus* were seen in two localities each. The mean ± SD number of species collected in ecologically unaltered areas was 2.4 ± 0.8, while the mean ± SD value for altered areas was 12 ± 2.6.

Breeding sites. Specific analyses of breeding sites near the Balbina hydroelectric dam were carried out because of the presence of black water (Uatuma River) and muddy water (Pitinga River). In the black water, the temperature varied between 25°C and 27°C. In the muddy waters, on the other hand, temperatures were between 21°C and 24°C. The pH was similar at all the study sites and was approximately 4.5.[12] The Urucu River in Coari, with black waters, has a less acidic pH of 5.0. The pH in the muddy breeding sites around Ariquemes, was between 7.0 and 8.0. Breeding sites with lower pHs had fewer anopheline larvae.

At Balbina, only the species *An.* oswaldoi and *An. mediopunctatus* were collected in these unaltered rivers. These two species have shown a tolerance to environmental vari-

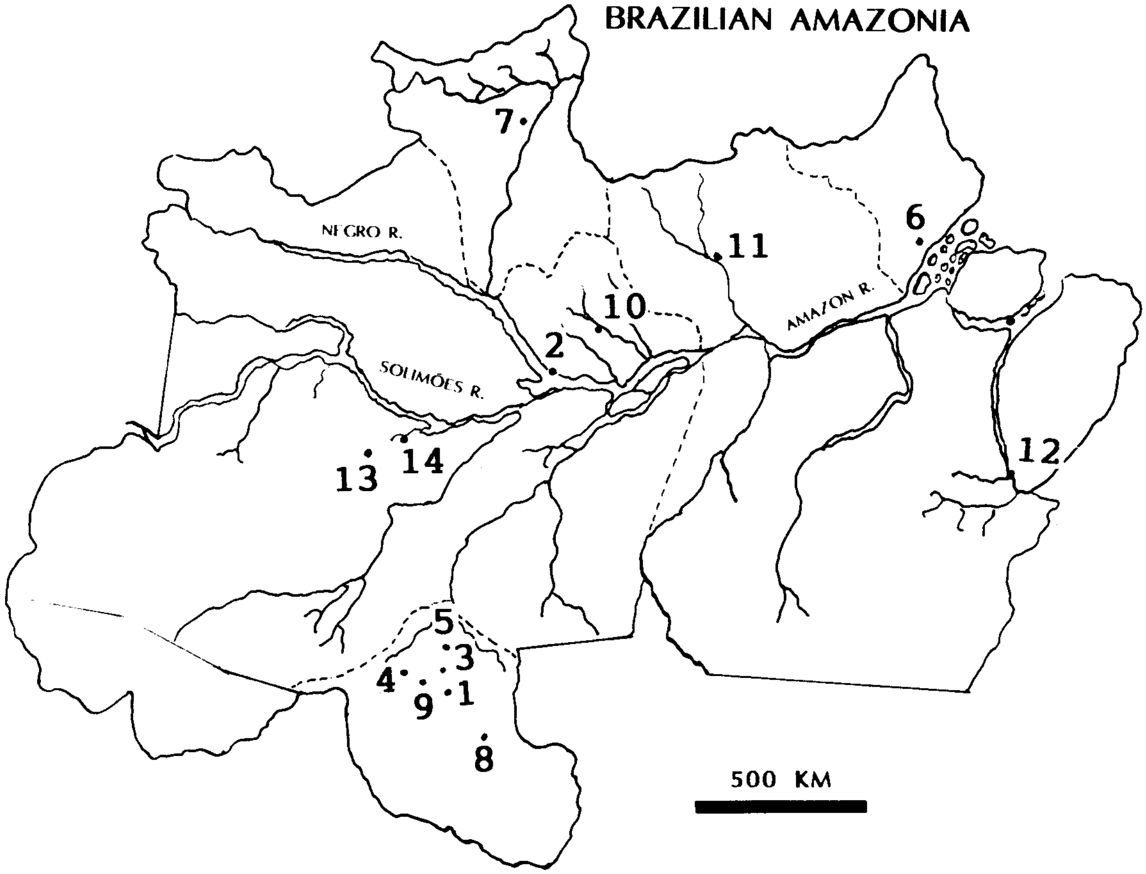

FIGURE 2. Study sites. Localities: 1 = Urban Ariquemes; 2 = Manaus; 3 = Ariquemes; 4 = University; 5 = Machadinho; 6 = Amapa's Garimpo; 7 = Maracá; 8 = Ji-Paraná; 9 = Samuel; 10 = Balbina; 11 = Porteira; 12 = Tucurui; 13 = Urucu Camp; 14 = Urucu-Coari River.

ations in pH, temperature, and nutrients.[13] A nearby locality (Mucuim), which had already been altered by human activities, showed a greater diversity: *An. oswaldoi* (71 larvae), *An. mediopunctatus* (10), *An. nuneztovari* (126), *An. rangeli* (1), *An. trianulatus* (3), and *An. benarochi* (2).

Species distribution and incidence. Some of the localities studied in Amazonia showed differences in both species distribution and incidence (Table 3). The urban area in Ariquemes showed the highest larval index (34.7 larvae/dipping) while rural Ariquemes had an index of 6.2 larvae/dipping. Some other localities, e.g., the city of Manaus, maintained an almost constant larval index over a three-year period, which was independent of the control efforts (temporary measures such as cleaning streams and chemically treating breeding sites). The indices for Manaus were 6.8 during 1993, 7.2 in 1994, and 7.0 in 1995, but the index increased considerably during 1996 to 12.0 in the rural invaded areas that had cattle ranches and fish farms. Larval collection indexes in the Urucu-Coari camp decreased in three consecutive years (1993 index = 1.9, 1994 = 1.1, and 1995 = 0.01. Collections along the Urucu River at the end of the 1996 rainy season gave an index of 19.6 larvae/dipping. Some of these collections were in unaltered areas and others were in areas where isolated settlers had engaged in farming activities and had thereby accidentally created better conditions for anopheline development.

The incidence of adult anophelines in Tucurui increased from 25.6 mosquitoes/person/hr when dam construction started to 53.3 mosquitoes/person/hour after the formation of a lake. This index stabilized at 3.65 mosquitoes/person/hr five years later after settlement by human populations. The predominant species all through the studies was *An.* nuneztovari. The malaria cases among workers and the local population in Tucurui were not as numerous as in rural Ariquemes, which had an adult mosquito index of 30.6 mosquitoes/person/hr and an abundance of *An. darlingi.* Maraca, an ecologic reserve, had the smallest anthrophilic mosquito index (0.06 mosquitoes/person/hr).

Table 4 shows the variation in the adult anopheline population indexes before, during, and after ecologic alterations. They ranged from 0.51 to 3.13 mosquitoes/person/hr before human interventions and from 1.5 to 462 mosquitoes/person/hr after these interventions. In Rondonia State, before the Samuel hydroelectric station was built, collection data show differences between the two sides of the white water Jamari River and also between the edge of the forest and its interior. Near the right river bank, collections from seven sites in the area of the dike revealed five anopheline species and an index of 0.32 mosquitoes/person/hr. Near the left bank, which was still unaltered but may have been affected by proximity to a highway (BR-364), we found one additional species, *An. darlingi.* The incidence index was 28 mosquitoes/person/hr. At the edge of the forest the index was 3.13 mosquitoes/

TABLE 1
Fourteen localities, collection dates, areas, and sites in the study*

Locality	State	Date	Areas L	Areas A	Sites L	Sites A
Urban localities						
1) Ariquemes	RO	8/84–6/85	8	10	56	166
2) Manaus	AM					
Year 1		1/93–12/93	–	–	110	137
Year 2		1/94–12/94	–	–	174	281
Year 3		1/95–12/95	–	–	153	129
Year 4		1/96–8/96	–	–	165	104
Rural localities						
3) Ariquemes	RO	8/85–11/88	–	2		
4) University	RO	2/87–7/87	–	–	43	49
5) Machadinho	RO	6/86–12/87	–	–	45	36
Open mine exploration						
6) Amapa's garimpos	AP	9/89–10/89	–	11	–	84
Ecologic reservation with invasions						
7) Maraca	RR	5/87–6/88	–	–	–	36
Hydroelectric dam construction						
8) Ji-Parana	RO	8/87–9/87	5	5	40	21
9) Samuel	RO	9/85–10/86	–	9	–	764
10) Balbina	AM					
Pre		11/83–9/87	14	14	337	242
>5 years						
11) Porteira	PA	10/85–8/86	5	5	143	138
12) Tucurui	PA					
Pre		7/80–11/81	7	84	7	74
Post		12/84–5/85	–	9	–	310
>5 years		8/90–10/90	–	–	–	86
Oil and gas exploration area Urucu-Coari						
13) Camp	AM					
Year 1		4/93–12/93	–	–	180	225
Year 2		1/94–12/94	–	–	518	297
Year 3		1/95–3/95	–	–	272	60
14) Urucu River		5/96–7/96	–	–	9	13

* L = larvae; A = adults; RO = Rondonia; AM = Amazonas; – = no collections were attempted; AP = Amapa; RR = Roraima; PA = Para.

person/hr and within the primary forest it was 2.6 mosquitoes/person/hr.

A very different situation was found at the Porteira Falls on the Cachorro River in Para State, where *An. darlingi* was present with an index of 53 mosquitoes/person/hr and only a single specimen of *An. oswaldoi* was captured. The diversity in this locality was very low, being basically represented by a single species.

In another natural environment at Balbina, before the construction of the hydroelectric dam, a low density of anophelines (0.51 mosquitoes/person/hr at 62 collection sites) and also a low diversity (two species) were noted. However, in areas where agricultural activities were taking place, the density and diversity were higher (1.5 mosquitoes/person/hr at 13 sites and seven species).

At Tucurui southeast of a reservoir after it was filled, 462 mosquitoes/person/hr were collected at 17 sites. The predominant species was *An. nuneztovari,* which represented 90% of the total.

At the hydroelectric stations of Tucuruí and Balbina, some species of the subgenus *Nysorrhynchus* colonized altered environments, becoming abundant or even dominant (e.g., *An. nuneztovari* and *An. triannulatus*). On the other hand, some species became rare or disappeared (such as *An. intermedius*). A third situation occurred at Tucurui with regard to *An. argyritarsis,* which was not found before the lake formed, was encountered after the lake was filled, but disappeared five years later. *Anopheles braziliensis,* which had been absent in the area, was collected there five years after the filling of the lake. In studies conducted at Balbina five years after the lake was filled, 11 species were found and the dominant one was *An. nuneztovari.*

The situation with regard to anopheline populations during the construction of the Amazonian highway BR-174 (Manaus-Boa Vista) is shown in Table 5. At the beginning of construction in 1965, *An. nuneztovari* coexisted with *An. darlingi* at densities of 3.25 and 19 mosquitoes/person/hr, respectively. Observations during 1994 showed an intradomiciliary index for *An. nuneztovari* of 1.71 mosquitoes/person/hr and a peridomiciliary index of 6.42 mosquitoes/person/hr. The corresponding figures for *An. darlingi* were 0.125 and 0.449, which indicated a reduction in the density of the principal vector for malaria.

Seasonality and feeding preferences. Variations of monthly species indexes are shown in Figure 3. In the rural zone of Ariquemes, anophelines were collected simulta-

TABLE 2

Larvae and adult anopheline species found in natural environments and altered localities*

Species	Nonaltered					Altered localities													
	4	7	9	10	11	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Subgenus Anopheles Meiges, 1818																			
Anopheles mattogrossensis Lutz & Neiva, 1911			X			X	X	X		X	X				X	X	X	X	X
peryassui Dyar & Knab, 1908						X		X		X	X	X	X	X	X				
Pseudopunctipennis Theobald, 1921													X	X					
Subgenus *Arribalzagia* Theobald, 1903																			
Anopheles apicimacula Dyar & Knab, 1906		X																	
intermedius (Peryassu, 1908)	X					X	X						X			X	X		
mediopunctatus (Theobald, 1903)					X		X	X		X	X	X	X	X	X	X	X	X	X
minor Lima, 1929								X											
shannoni Davis, 1931									X	X			X	X	X				
Subgenus *Lophodomyia* Antunes, 1937																			
Anopheles squamifemur Antunes, 1937															X				
Subgenus *Nyssorhynchus* Blanchard, 1902																			
Anopheles albitarsis Lynch Arribalzaga, 1878	X		X		X	X	X	X		X	X		X	X	X	X	X	X	X
argyritarsis Rovineau-Desvoidy 1827			X			X		X		X	X	X		X	X			X	X
benarrochi Gabaldon, Cova & Lopez, 1941						X		X						X					
braziliensis (Chagas, 1907)		X	X			X	X	X	X	X	X	X			X			X	
darlingi Root, 1926						X	X	X		X	X	X	X	X	X	X	X	X	X
deaneorum							X		X						X				
evansae (Brethes, 1926)						X	X	X			X			X		X	X		
galvaoi Causey, Deane & Deane, 1943						X		X		X	X								
noroestensis Galvão & Lane, 1937																	X		
nuneztovari Gabaldon, 1940				X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
oswaldoi (Peryassu, 1922)				X		X	X	X	X	X	X	X	X	X	X	X	X	X	
rangeli Gabaldon, Cova & Lopez, 1941						X		X	X	X			X	X		X	X		
rondoni (Neiva & Pinto, 1922)																X	X		
strodei Root, 1926						X		X		X		X							
triannulatus (Neiva & Pinto, 1922)						X	X	X	X	X	X	X	X	X	X	X	X		X
Subgenus *Kerteszia* Theobald, 1905																			
Anopheles neivai Howard, Dyar & Knab, 1912														X	X				
Subgenus *Stethomyia* Theobald, 1902																			
Anopheles nimbus (Theobald, 1902)												X							X
TOTAL (number of species)	2	2	4	2	2	15	11	16	7	14	12	10	11	14	14	11	12	8	8

* For the names of the localities, see Table 1.

TABLE 3
Anophelines collected in the Brazilian Amazon*

Localities	State		Person hr		Larvae		Adults	
			L	A	Total	Index	Total	Index
1) Ariquemes-urban	RO		224	664	7,778	34.7	5,436	8.2
2) Manaus-urban	AM	1993	440	1,096	2,985	6.8	3,073	2.8
		1994	696	2,248	5,051	7.2	2,533	1.1
		1995	612	1,032	4,287	7.0	2,333	2.3
		1996	1,320	832	7,394	5.6	1,107	1.3
3) Ariquemes-rural	RO		236	424	1,468	6.2	12,985	30.6
4) University-rural	RO		172	196	97	0.6	827	4.2
5) Machadinho-rural	RO		188	148	938	5.0	714	4.8
6) Garimpos	AP		–	336	–	–	2,189	6.51
7) Maraca	RR		244	144	1,161	4.76	2,334	0.06
8) JiParana (HDA)	RO		160	84	685	4.28	731	8.7
9) Samuel (HDA)	RO		532	3,056	479	0.9	6,684	2.2
10) Balbina (HDA)	AM		1,348	968	2,885	2.14	3,011	3.1
11) Porteira (HDA)	PA		572	552	1,359	2.37	8,627	15.6
12) Tucurui (HDA)	PA	Pre	336	296	6,533	19.44	7,598	25.6
		Pos	–	1,240	2,548	–	66,061	53.3
		>5 years	–	344	–	–	1,257	3.65
13) Urucu-Coari Camp	AM	1993	720	900	1,351	1.9	868	0.9
		1994	2,072	1,188	2,384	1.1	246	0.2
		1995	1,088	240	12	0.01	6	0.02
14) Urucu River	AM	1996	36	52	707	19.6	3,436	66.1

* L = larvae; A = adults; Larval index = mean number of larvae per dipping; Adult index = index of mosquitoes captured divided by person hr; RO = Rondonia; MA = Amazonas; AP = Amapa; – = no collections were attempted; RR = Rorarima; HDA = hydroelectric dam area; PA = Para.

neously on animals and humans to evaluate their level of anthropophilia and zopophilia. The species *An. strodei, An. galvaoi,* and *An. peryassui* were highly zoophilic, being found on both cattle and pigs, but showing a preference for cattle. *Anopheles darlingi* confirmed its reputation for anthropophilia; 59% of those captured were trying to feed on humans or were resting on nearby bushes (Figure 4).

Hours of maximum activity. Figure 5 compares the patterns of activity in Ariquemes (highways BR-319 and 422) and Tucurui from 6:00 PM to 6:00 AM.

Resting places. In the rural area of Ariquemes, 1,974 anophelines were collected and only 18% were found inside houses. However, 99% of these were *An. darlingi.* The other species predominated outside the houses. The highest percentage of *An. darlingi* endophilia was seen at the Machadinho colony in Rondonia where of 415 specimens collected at 37 sites, 32% were intradomiciliary.

Along highway BR-364, 26% of 1,546 anophelines were captured in the interior of houses. At Tucurui, in agricultural colonies situated at the edge of the lake, endophilia was 40% among 3,006 anophelines captured. *Anopheles nuneztovari* was the species that predominated and represented 85% of the total that was caught inside houses.

In the residential areas of the hydroelectric stations of Samuel, Balbina, and Tucurui, the houses are of better quality and have screened windows and tiled roofs. In these areas, only 0.6% of 500 *An. darlingi* captured were intradomiciliary. The same situation was observed at the oil and gas camp at Urucu-Coari.

Vertical distribution. Observations on the behavior of *An.* darlingi were carried out in the vegetation around human dwellings at Ariquemes. Mosquito collections were separated into 0–1 meters (low) and greater than 1 meter (high). A total of 804 mosquitoes were obtained, 303 at higher levels and 501 at lower levels, indicating a marked preference for low-level vegetation.

***Plasmodium* in anophelines**. *Anopheles darlingi, An. braziliensis, An. nuneztovari, An. triannulatus,* and *An. oswaldoi*

TABLE 4
Comparison of anopheline populations before and after human intervention: number of sites, hours, species, mosquitoes, and incidence*

	Number of				Index
	Sites	Hours	Species	Mosquitoes	Mosquitoes/ person/hr
Before human interventions†					
Balbina (AM)	62	248	3	126	0.51
Jamari River, right bank (RO)	7	28	5	9	0.32
Samuel, inside the forest (RO)	43	172	–	120	0.7
Samuel, forest border (RO)	50	200	–	626	3.13
After human interventions†					
Balbina, agronomic area (AM)	13	52	7	78	1.5
Jamari River, left bank (RO)	5	20	6	561	28
Cachoeira Porteira (PA)	38	152	1	8,047	53
Tucurui (PA)	17	68	2	31,430	462

* AM = Amazonas; − = no collections were attempted; RO = Rondonia; PA = para.
† For collection dates, see the Materials and Methods.

TABLE 5
Anopheles populations detected during the construction of the Manaus-Boa Vista Highway, BR-174*

Houses along BR-174	Year	*An. darlingi*	*An. nuneztovari*
All collections	1965	19	3.25
Intradomiciliary	1994	0.125	1.71
Peridomiciliary	1994	0.449	6.42

* Values are mosquitoes/person/hr.

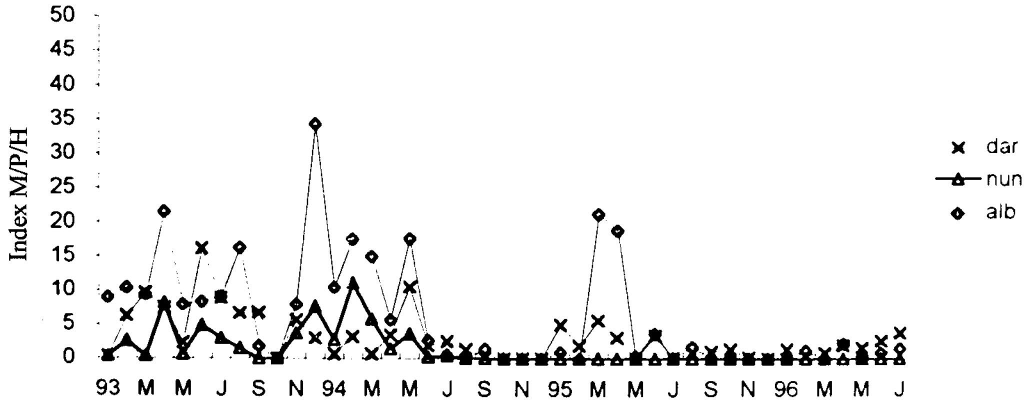

FIGURE 3. Seasonality of adult anophelines in Manaus from 1993 to 1996. M/P/H = mosquitoes/person/hour; dar = *darlingi;* nun = *nuneztovari;* alb = *albitarsis.*

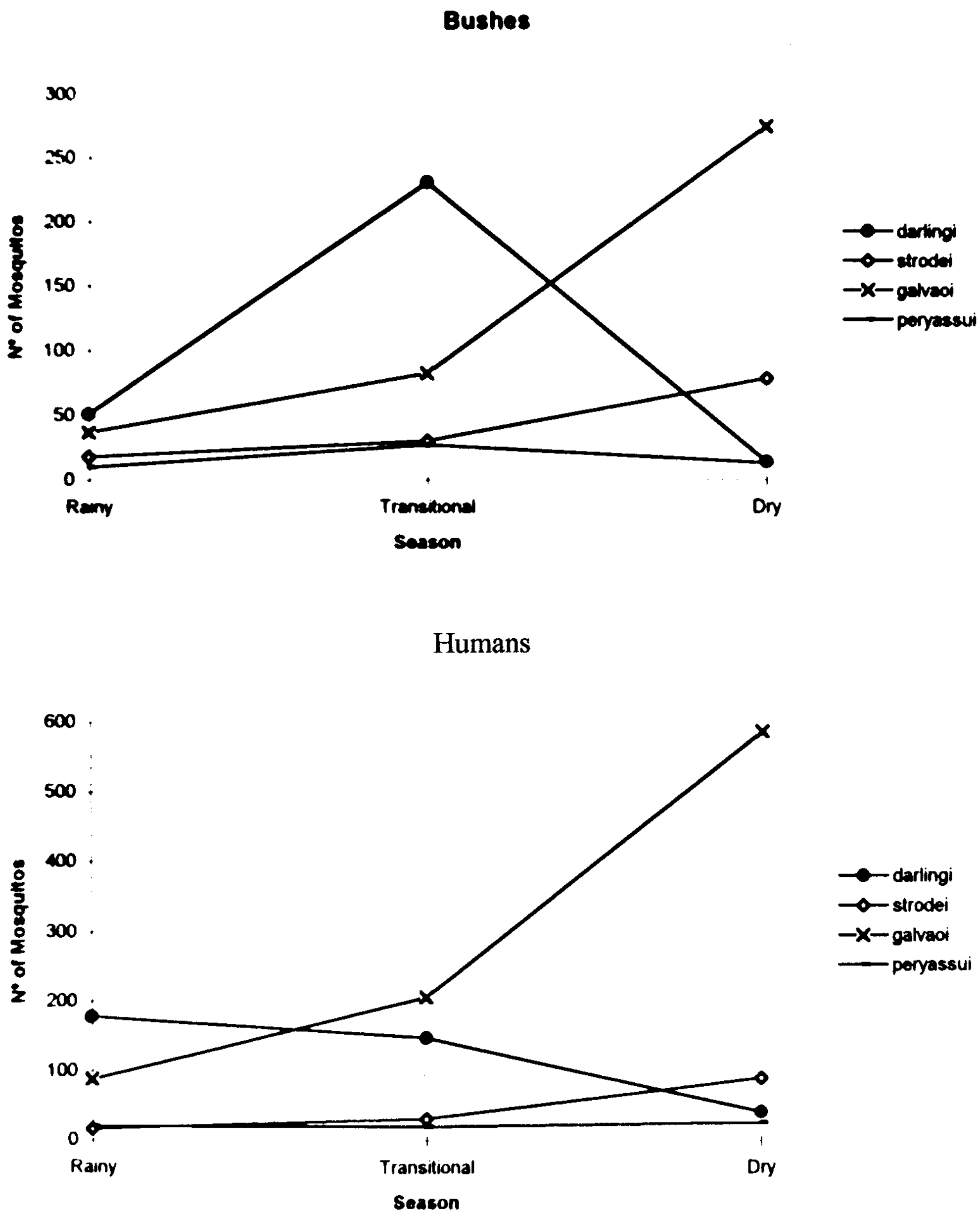

FIGURE 4. Feeding preferences of some anophelines during 84 collection hours in the rainy, dry, and transitional seasons.

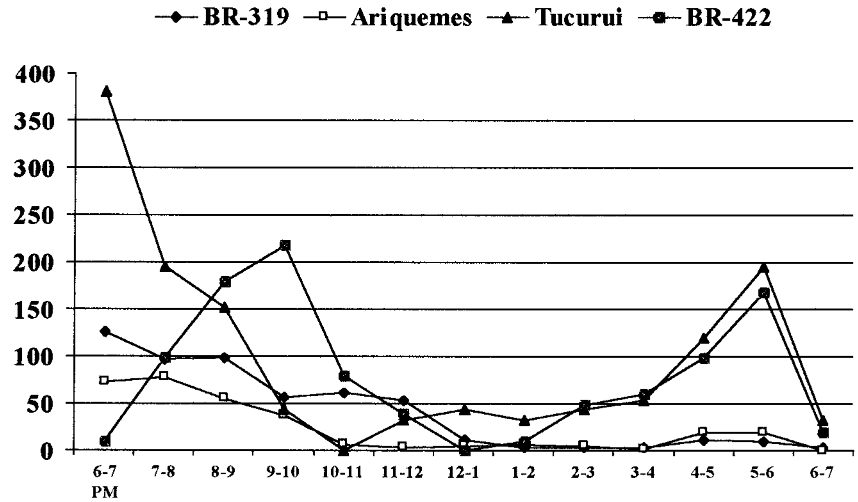

FIGURE 5. Comparison of feeding activities of *Anopheles darlingi* in four different sites in the Brazilian Amazon from 6:00 PM to 6:00 AM. Values on the y-axis are the number of mosquitoes/person/hour.

were shown to be positive for *P. falciparum* in an immunoenzymatic test using monoclonal antibodies against circumsporozoites (Table 6). The same test results were positive for *P. vivax* in *An. braziliensis, An. darlingi, An. galvaoi, An. nuneztovari, An. strodei*, and *An. triannulatus* (Dutary Thatcher B, Tadei WP, unpublished data). Figure 6 shows the relationship between the increases in the density of *An. darlingi* and the increases in the number of malaria cases in Manaus that occurred 30 days later.

Species complex. The description of cryptic species complexes in the genus *Anopheles* is contributing to the separation of closely related forms that may be involved in the transmission of malaria.[14] At this time in Amazonia, *An. nuneztovari*, An. darlingi, and *An. albitarsis* are of special interest[15–19] because ELISA data show that these species are infected with *Plasmodium* and they are collected close to human habitation.

Data on alloenzymes of populations of *An. nuneztovari* from the Brazilian Amazon and from Colombia showed genetic differentiation at an interspecific level. It was proposed that the taxon *An. nuneztovari* is composed of at least two cryptic species whose speciation occurred allopatrically.[20] Studies of populations of *An. darlingi* in regard to behavioral patterns, chromosome morphology, alloenzymes, and cuticular hydrocarbons have not shown levels of differentiation that could suggest subpopulations of this species.[21, 22] A high level of variability is found in this species, which give it sufficient adaptive capacity to be able to exploit the different habitats within its extensive geographic range.

TABLE 6
Anopheles species infected with *Plasmodium*

Amazonian species	Infection detected by: Microscopy	ELISA: *P. vivax*	ELISA: *P. falciparum*
An. albitarsis	x		
*An. aquasalis**	x		
An. braziliensis	x		
An. darlingi†	x	x	x
An. galvaoi		x	
An. nuneztovari		x	
An. oswaldoi			x
An. strodei	x	x	
An. triannulatus		x	

* Principle vector on the coast.
† Principle vector in the interior.

DISCUSSION

Some 54 species of *Anopheles* are present in Brazil and 33 of these have been found in Amazonia. Most breeding sites were found in open areas in creeks with running water, in shaded areas with stagnant waters, in aquatic vegetation such as *Pistia* sp., *Ludwigia, Cabomba, Tonina, Miconia,* and *Rollinia,* and in small, open, sunny streams with sandy bottoms. Breeding also occurred in stagnant water left after deforestation, on fish farms, and in puddles left after the river level dropped. At Balbina, 19 different species of diatoms were identified and most were of the family Eunotiaceae. The presence of acidophilic and oligrotrophic algae indicates an environment that is poor in nutrients.[13] Localities altered by human activites showed a greater diversity of anopheline species, which is probably caused by agricultural activities that provide additional nutrients to the area.

The effort to obtain data on anopheline population dynamics before, during, and after ecologic alterations demonstrated an increase in mosquito density and diversity in localities with human interventions. At most of the localities studied, population density was low within primary forest and tended to remain low even after nearby ecologic alteration occurred. In the case of hydroelectric dams, density has a direct relationship to the formation of lakes since the growth of macrophytes on the surface provides excellent breeding sites for anopheline mosquitoes. During construction of highways, the decrease in the density of *An. darlingi* near houses may be explained by deforestation, which increased the distances between residences and the forest and exposed breeding sights to sunlight. These factors apparently favored *An. nuneztovari.*

Aggressive and profound improvements in the infrastructure of Manaus, such as the leveling of land and modern

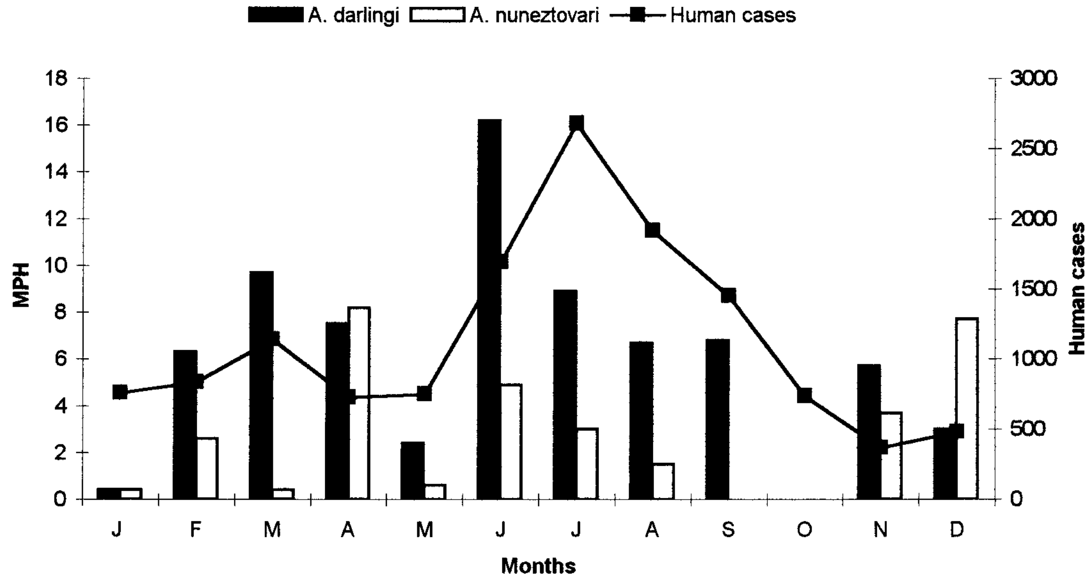

FIGURE 6. Relationship between the number of human malaria cases and densities of *Anopheles darlingi* and *An. nuneztovari*. MPH = mosquitoes/person/hour.

urbanization, eliminated *An.* darlingi from the city by 1976. However, the rapid expansion of the Manaus suburbs in recent years provided conditions for re-establishing contact between the human population and the principal vector, which was restricted to the surrounding jungle. *Anopheles darlingi* invaded the new periurban areas in 1988 and colonized the city once again. During 1993, an epidemic of malaria affected 23,189 people. Malaria cases continued to be reported from the periurban areas of the city in 1998.

Anopheles darlingi is the principal vector of malaria in Amazonia because it is highly anthropophilic.[23–25] Precipitin tests indicated a definite preference of this species for humans.[26] This species is able to maintain malarial endemicity even when densities are low.[27] Sporozoites found by dissection and positive immunologic test results for malaria are more frequent in this species than in the others.[28]

The hours during which anophelines attempt to feed on blood may vary depending on 1) the area (whether urban or rural), 2) the density of the anopheline species, 3) the density of other species of culicids, 4) the time of the year (rainy or dry), 5) the distance between the houses and the forest, and 6) the presence or absence of other hosts.

In both urban and rural areas, *An. darlingi* fed all night but had a peak of activity at the beginning of the evening and another at dawn. The early morning peak was less pronounced in the other areas studied: Tucurui, highway BR-319 (Rondonia-Amazonas), and highway BR-174. In the urban zone of Ariquemes, this anopheline was more frequent during the first period (6:00–8:00 PM). The other anophelines are mainly crepuscular with intense feeding activity in the early hours of the night. In these species, there may or may not be a small early morning peak; *An. nuneztovari*, *An. oswaldoi*, *An. triannulatus*, *An. albitarsis*, and *An. braziliensis* fed actively during the first period (6:00–8:00 PM) and ceased feeding around 9:00 PM. More detailed observations made on *An. nuneztovari* at 15-min intervals throughout the night showed that the most intense feeding peak occurred between 7:00 PM and 7:30 PM. Patterns of feeding activity appear to be related to population densities. When densities are low, feeding is restricted to the early hours of the night and terminated sometime between 8:00 PM and 9:00 PM.

In the region of Tucuruí after the lake was filled, species of *Mansonia* became very abundant. In these circumstances, the main peak of anopheline feeding activity was found earlier in the evening (6:00 PM).

Our data show a reduction in the density of populations of *An. darlingi* during the course of the collections and a relatively abrupt increase in the density of other species, such as *An. galvaoi*, *An. peryassui*, and *An. strodei.* Figure 3 shows that the number of anophelines collected was 5,676, and the decrease in *An. darlingi* and the increase in the other species was evident.

In Roraima and Amapa States where agriculture is practiced on low-lying, periodically flooded land, there are numerous breeding sites near the farms and high anopheline densities. Under such conditions, the dry and rainy seasons do not have much of an effect on anopheline populations and species of *Anopheles* feed actively all night long.

Recent colonizations by small farmers along highways have always resulted in deforested areas between the access road and the primary forest. The houses are located along the roadway not far from the forest. In such situations, *An. darlingi* establishes a night and day feeding pattern with individual females returning to the forest after taking a blood meal. As the deforestation continues and the houses are located progressively farther from the woods, the activity cycle of *An. darlingi* becomes nocturnal and diurnal feeding ceases. The differences observed between old and new farms leads one to believe that 3–5 years may be required for this cycle to become established.[5]

For *An. galvaoi* and *An. peryassui*, species that showed a preference for cattle, during colder periods (June–August) their feeding activity becomes intense during the first hour of the night.

The timing of feeding activity varies from place to place especially in areas that have been altered by human intervention. Time of the year and climatic conditions may also modify feeding. Observations made during the 1940s in Amazonia[29] indicated that *An. darlingi* was the principal vector of malaria and that transmission occurred at night within houses. To see if this assumption could be verified, we made intradomiciliary and peridomiciliary collections of anophelines in several areas. It was found that the anophelines penetrated the houses by way of low cracks in the walls and did not land on the DDT-treated interior surfaces. In the peridomiciliary zone, their preferred landing or resting sites were on nearby bushes up to about a meter high. From there, they could enter the houses, take a human blood meal, usually from the lower legs, and leave with little probability of coming into contact with residual insecticides.[25] A knowledge of the degree of exophilia and endophilia is necessary to determine the control strategy to be used. In Venezuela, for example, *An.* nuneztovari is exophilic and spraying the interiors of houses with residual insecticides turns out to be inefficient.[30] These habits are an example of the mosquito's ability to adapt to altered environmental conditions.

Within the subgenus *Nyssorhynchus*, the species involved in the transmission of malaria as indicated by dissection are *An. darlingi, An. albitarsis, An. aquasalis, An. braziliensis,* and *An. strodei.*[23] *Anopheles darlingi* is the principal vector in the interior of Amazonas State, while *An. aquasalis* is the vector in brackish waters along the coast in arid zones of the northeast in Belem, Para State and in Amapa State. The other species are occasional vectors and are found infected only where *An. darlingi* has initiated an outbreak.[31, 32]

Anopheles nuneztovari is a neotropical species of importance in the transmission of malaria in the Americas. It is found in northern South America and in eastern Panama. Studies of its chromosomes by means of molecular markers made possible the identification of different populations of this species. Data on polymorphism of the chromosomes indicate that three cytotypes are present.[33] Cytotype A shows a standard pattern, with a fixed inversion on the X chromosome and a distribution covering all of the Brazilian Amazon. Cytotype B has an inversion on chromosome II (2La), and is found in Venezuela southeast of the Andes. Cytotype C shows a complex of inversions on chromosome II (2Lc and 2Ld) included in 2Lb and a well-defined chromocenter. It is found in western Colombia and Venezuela. Studies on ribosomal and mitochondrial DNA of populations of *An. nuneztovari* from Venezuela, Brazil, Suriname, and Bolivia did not show enough differences to suggest the presence of a species complex.[19, 34]

Amazonia is vast and ecologically complex. Each habitat has special characteristics that make generalizations difficult. However, there are always some species of *Anopheles* that are able to survive and populate the varied ecologic niches. Human interventions in the forest promote contacts between humans and vectors and may rapidly produce disease foci.[5] Immigrants to Amazonia usually come from regions that are free of malaria and thus have no acquired immunity to the disease. Since the vectors of malaria are able to adapt to different environmental conditions, it is necessary to study each area before selecting control strategies. Tadei[35] indicated that some of the important factors that should be considered are the total number and species diversity of anophelines in the area, the degree of contact that exists between humans and vectors, the susceptibility to infection by *Plasmodium* of the anopheline species present, and the proportion of mosquitoes that are infected and long-lived.

Female anophelines show different degrees of importance in the transmission of malaria. Nulliparous and young females do not transmit malaria but tend to predominate during the two anopheline activity peaks. Oviparous females that can transmit malaria are found to be present in variable percentages depending on the hour.[35, 36] Our observations in Manaus demonstrated that nulliparus anopheline females were active between 6:00 PM and 9:00 PM while oviparus ones are active all night long, but at a low density ($\chi^2 = 6.005$, degrees of freedom = 1, $P < 0.05$). Each blood meal increases the probability that a mosquito will become infected and therefore older anophelines represent a greater risk. Older populations have a larger number of individuals infected but the population size is smaller.

The feeding preferences of mosquitoes such as *An. strodei, An. galvaoi,* and *An. peryassui*, which are extremely zoophilic could provide a natural biologic barrier when animals are near the house, as seen in the Rondonia studies (Figure 4). In Amazonia, the anophelines demonstrate a pronounced exophilia and therefore control methods that only include intradomiciliary spraying and the treatment of infected people are insufficient to reduce the transmission rate of malaria.[23–25] It is important to inform local inhabitants as to the habits of anopheline mosquitoes.

Studies of infection of *Anopheles* species with *Plasmodium* suggest that *An. darlingi* may be responsible for maintaining malaria in human populations. A reduction in the population density of *An. darlingi* in a particular geographic area can sometimes bring about the disappearance of malaria.[4, 23, 32, 35]

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