

THE EFFECT OF DEFORESTATION ON THE HUMAN-BITING RATE OF *ANOPHELES DARLINGI*, THE PRIMARY VECTOR OF FALCIPARUM MALARIA IN THE PERUVIAN AMAZON

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Abstract. To examine the impact of tropical rain-forest destruction on malaria, we conducted a year-long study of the rates at which the primary malaria vector in the Amazon, *Anopheles darlingi*, fed on humans in areas with varying degrees of ecological alteration in the Peruvian Amazon. Mosquitoes were collected by human biting catches along the Iquitos-Nauta road at sites selected for type of vegetation and controlled for human presence. Deforested sites had an *A. darlingi* biting rate that was more than 278 times higher than the rate determined for areas that were predominantly forested. Our results indicate that *A. darlingi* displays significantly increased human-biting activity in areas that have undergone deforestation and development associated with road development.

INTRODUCTION

Deforestation of the Amazon rain forest has greatly changed the distribution and abundance of a multitude of tropical faunal and floral species. It has been postulated that arthropod disease vectors have also been affected, in turn altering disease patterns.¹⁻³ To explore this association, we examined the human-biting rate and distribution of the malaria vector *Anopheles darlingi* by the degree of deforestation and ecological alteration in the northern Peruvian Amazon, in conjunction with *Anopheles* larval studies and human malaria prevalence studies. In this paper, we will be presenting our findings pertaining to the adult vector. Malaria prevalence in northeastern Peru (Loreto district, 348,177 km²) was high prior to 1965 but dropped dramatically with the eradication efforts of the late 1960s. Until the early 1990s, malaria prevalence remained very low, with 123 cases of falciparum malaria (0.4 cases per 1,000 population) and 518 cases of vivax malaria (1.7 per 1,000) reported in Loreto in 1992. However, in 1997 more than 120,000 cases of malaria (343 per 1,000) were reported, 45% of which (154 of 1,000) were due to *Plasmodium falciparum*.

The history of malaria is intimately tied to the history of Iquitos. In 1842, Iquitos was a mere village of 200 inhabitants; this number had increased to 14,000 in 1903 owing to the rubber boom.⁴ After this golden era came to an end during World War I, cattle ranching, gold mining, and logging became the dominant industries.⁴ In the second half of the 20th century, oil mining and drug trafficking generated much income in the region. In the 1990s, the population growth in the Amazon was enhanced by policies encouraging the migration of people to Iquitos from Andean regions, similar to Brazil's relocation efforts in the 1980s.⁵ Civil unrest in the Andes due to the Shining Path and the military contributed further to

this migration.⁶ As a consequence of these events, the population of Iquitos numbered 305,514 inhabitants in 1993.⁴

Currently, the majority of the population of Loreto (population, 351,940) engages in subsistence-scale agriculture (mainly growing plantain and cassava root), fishing, and hunting. The former accounts for most of the deforestation in the study region.⁴ This stands in contrast to the Brazilian Amazon, where 70% to 75% of all deforestation is due to large- and medium-scale cattle ranches.⁷ Other important sources of employment in the study region are lumber milling, canning industries, poultry, cattle, and fish farming.⁸ These activities and the population explosion have contributed to deforestation, which took place at a rate of 4,257 hectares per year between 1983 and 1995.⁹ The construction of a 95-km, partially paved road between the Amazon port cities of Iquitos and Nauta has enabled expansion into areas that were previously difficult to access, leading to a human population growth rate of 7.4% along this road (in contrast to Iquitos city's annual growth rate of 3.4%). Deforestation has followed the contours of the road, widening every year due to land being cleared for farming along the margins.

An increase in malaria cases was observed in another part of the Amazon that had undergone similar ecological pressures. In Belém, Brazil, *Anopheles darlingi* reappeared in 1992 after its presumed elimination in 1968. The authors document the subsequent rise in malaria prevalence and hypothesize that the reinvasion of *A. darlingi* was due to the encroachment of the city on the surrounding forest.¹⁰

Several decades prior, a malaria epidemic ensued after forest had been cleared in Trinidad as a result of the vector *Anopheles bellator* invading the newly created habitat.¹¹ This report of changes in vivax malaria prevalence after forest clearing is one of the very few studies that illustrates the ecological chain of events leading to a malaria epidemic and its resolution. In a study of *A. darlingi* vector ecology, Tadei and colleagues¹² sampled *Anopheles* larvae and adults in 14 areas altered by human activity (e.g., hydroelectric dams and roads) as well as 5 unaltered areas in the Brazilian Amazon between 1993 and 1996. *A. darlingi* was encountered in 13 of the 14 altered areas, but in none of the unaltered sites, suggesting that certain types of human activity increase malaria risk.

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Here we set out to test the hypothesis that deforestation is positively associated with the *A. darlingi* human-biting rate in the Peruvian Amazon, an area that has experienced a recent upsurge in malaria. This relationship is possibly mediated by an increase in suitable breeding sites. *A. darlingi* is typically described as a sylvatic riverine species though it has also been observed in anthropogenic environments (e.g., irrigation canals, rice fields, pastures, roadside).¹³⁻¹⁶ Before 1991, no *A. darlingi* were captured in a study of mosquitoes in Loreto (except along the borders with Ecuador and Brazil).¹⁷ By 1995, however, *A. darlingi* had reemerged, becoming the most abundant *Anopheles* species collected in settlements.¹⁸ The reemergence of this species is of interest because it has been shown to be the most efficient vector for malaria in the Amazon Basin.¹⁹⁻²² Malaria parasite sporozoites have been found

in its salivary glands (an indicator of the mosquito's ability to infect its host) at rates significantly exceeding those seen in other *Anopheles* (natural sporozoite infection rates of 0.4% and 0.1% have been reported for *P. falciparum* and *P. vivax*, respectively, in Rondonia, Brazil).²³ It also occupies a large geographical range (from Belize to Bolivia) and preferentially feeds on humans.²⁴⁻²⁶

MATERIALS AND METHODS

Geography of study area. The study area is located in the northern Peruvian Amazon, spanning the Iquitos-Nauta road (3°S, 73°W) (Figure 1). The road extends for 95 km, 72 km of which had been paved by August 2001 (compared with 48 km in March 1999). The city of Iquitos lies at the northern-

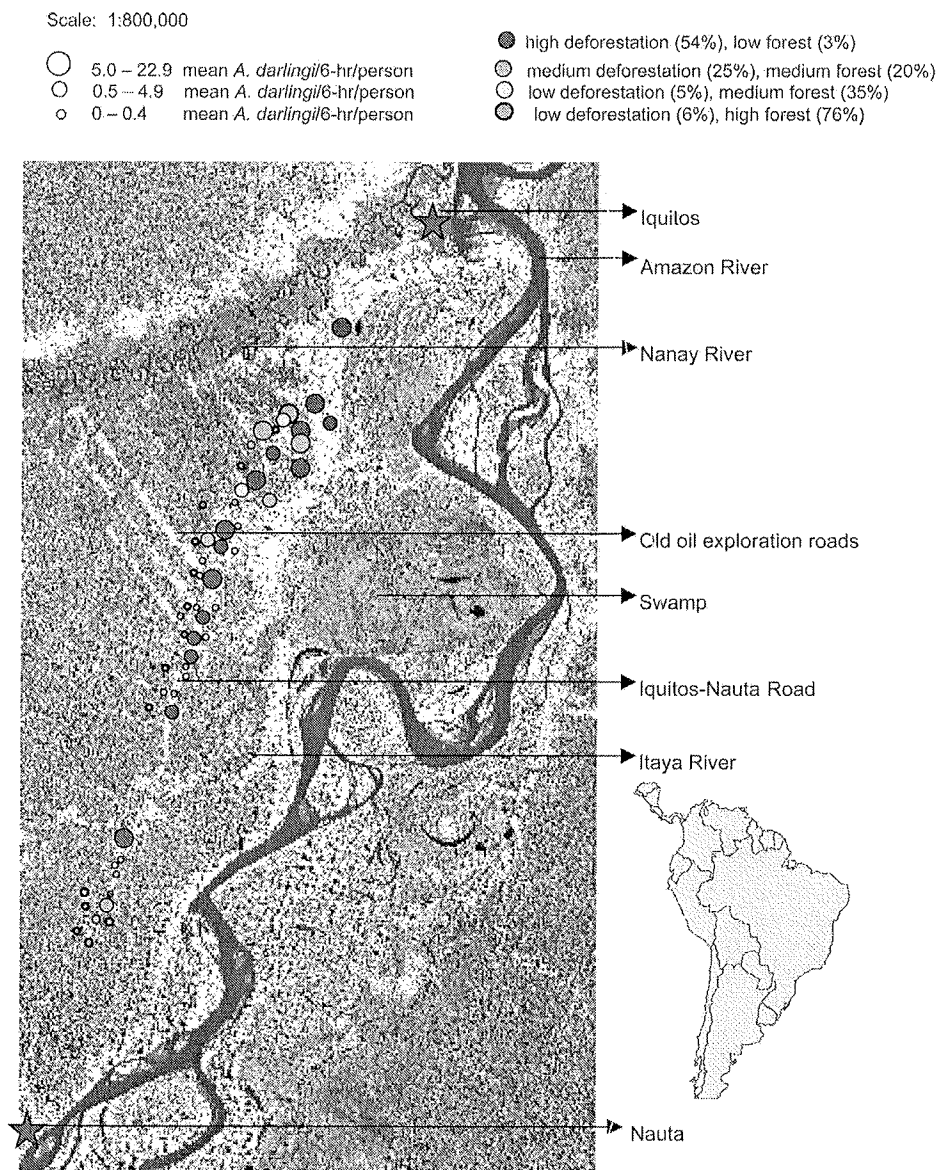


FIGURE 1. Satellite image of the study area with the 56 collection sites mapped with circles of varying sizes and colors. The size of the circle represents the mean number of *A. darlingi* collected per collection night (6 hours) per person, and the color of the circle represents the relative amount of forest surrounding the collection site. This figure appears in color at www.ajtmh.org.

most point of the road, surrounded by the Amazon River to the east, the Nanay River to the north, and the Itaya River to the south. At the southernmost end of the road lies the smaller Amazon port city Nauta (population, 15,000). The temperature fluctuates little during the year, remaining generally between 24°C and 28°C, with humidity ranging between 65% and 100%. There are several pronounced seasons: the rainy season, January through May or June; the dry season, July through December; and a short rainy period in November (“inviernillo,” or small winter). Interannual variability is considerable, however.⁸

Site selection and specifications. Fifty-six sites with varying degrees of deforestation were sampled once every 3 weeks from September 2000 through August 2001 (15 or 16 collections per site, for 56 sites, totaling 887 collections for the entire study). The necessary number of sites was determined using our December 1998 through June 1999 pilot study of *Anopheles* human-biting rates and deforestation in this region. To obtain a sampling representative of the different types of ecology in the region, we selected sites *a priori* by vegetation type and human population density (because the latter was considered a potential confounding factor). This selection process was accomplished by recording the GPS coordinates of all houses, and the number of inhabitants per house, from kilometer 18 to kilometer 48, and kilometer 55 to kilometer 70 along the Iquitos-Nauta road at the outset of the study. We excluded the region between kilometer 48 to kilometer 55 due to the presence of the Itaya river, which would have represented a distinct riverine ecology, inconsistent with the sites along the remainder of the road (based on pilot study results). This allowed us to calculate the coordinates of the center of each village along the road, weighted for the number of inhabitants per house. There were 12 distinct settlements (and thus village centers) at roughly 4-km intervals in this stretch. Next, using a 2000 Landsat Thematic Mapper 7 satellite image (U.S. Geological Survey, EROS Data Center, Sioux Falls, SD) of the area, we identified areas with predominantly forest, deforestation, and secondary vegetation growth surrounding the village centers. GPS coordinates of points lying within those forested, deforested, and secondary growth areas, but separated by at least 1 km from adjacent points, were then chosen for each of the 12 villages. Finally, we located these coordinates in the field and marked them as collection sites. For each of the 12 villages, a single house nearest to the village center was chosen, and a mosquito collection site was established outside the house (at 5–10 m from the entrance). Thus each village had one collection site in the village center, one in a deforested area approximately 1 km from the center, one in secondary vegetation, and one in a forested area. An additional four collection sites were selected in the more populous stretch between kilometer 7 and kilometer 21 (one site in each of four different village centers), as well as four more collection sites in the sparsely inhabited, mostly forested section of the road between kilometer 68 and kilometer 72. In total, there were therefore 14 clusters of sites, with 4 sites each.

The vegetation composition surrounding each site was determined using a 2001 Landsat Thematic Mapper 7 satellite image, which has a resolution of 30 × 30 m per pixel. The image was registered and geodetically corrected using paper maps of the region (National Imagery and Mapping Agency, Lima, Peru) (error is less than 1 pixel, or 30 m). The cloud

coverage in this image over the study area was 1.3%, which was low relative to other Landsat images taken during the study period. The pixels that represented cloud cover were recoded as data points for which landscape information was missing. The vegetation and landscape classes were then determined by means of unsupervised classification specifying 10 classes, and the vegetation or landscape type was assigned to each class using a subset of ground-truthed data (composed of global positioning system [GPS] coordinates with ecological observations made in the field).²⁰ The classified image was then assessed for accuracy by generating the percent agreement between the remainder of the ground-truthed data and the assigned vegetation classes. The percent agreement was 72%. The error may be attributable to registration, classification, and GPS error. Square grids of varying sizes were superimposed over each collection site using geographical information systems (ERDAS Imagine 8.5, Leica Geosystems GIS & Mapping LLC, Atlanta; ArcView 8.2 and ArcInfo 8.0, ESRI, Redlands, CA).

Different grid sizes were examined (200 × 200 m, 1 × 1 km, 2 × 2 km, 5 × 5 km, and 7 × 7 km) because the area that would influence *A. darlingi* behavior was unknown. The limit of 7 × 7 km was chosen because the maximum flight range reported for *A. darlingi* is 7 km.¹⁹ The percentage of each landscape class within the grids was calculated. In addition, the distances to the nearest edge of each of these features were determined. The human population density was ascertained by calculating the number of people within a 200-m, 500-m, 1-km, and 1.5-km radius from the mosquito collection site using georeferenced houses with the accompanying census data.

Three of the 10 landscape classes were collapsed into a single class, “forest.” “Forest” refers to closed-canopy, tall forest, and “varillal” is a naturally occurring forest growing on sandy soils that support vegetation of lesser height and a sparser canopy. “Secondary growth” refers to the successive vegetation after deforestation (approximately 15 years prior), and “shrub” is used here to refer to younger successive vegetation (arising approximately 5 years after deforestation). “Grass/crop land” may or may not be cultivated and maintained. “Bare surface” is used to indicate compacted soil, asphalt, concrete structures, and tin roofs. Both “deep water” and “shallow water” are bodies of water at least 20 × 20 m in size; the former has little sedimentation and the latter is either shallow or contains large amounts of sediment.

Mosquito collection method and identification. Landing catches were undertaken from 6 PM to midnight (12-hour collections were undertaken during the pilot study indicating that the *A. darlingi* in this region are unimodal with peak biting periods occurring between 9 and 11 PM). The collectors aspirated mosquitoes off their own legs for the first 50 minutes of each hour and rested for the remaining 10 minutes. Eight sites were sampled simultaneously, with one mosquito collector per site, for 7 consecutive days every 3 weeks. Mosquito collectors were rotated to eliminate bias due to individual variation among collectors and later included in the analysis to check for confounding. Collection sites in human settlements were located outdoors, 5 to 10 m from the selected house. Each site was sampled at 3-week intervals between September 2000 and August 2001 (16 collections total). Mosquitoes were identified morphologically using the 1994 key by Consoli and Lourenço-de-Oliveira.¹³

Statistical analysis. We tested the hypothesis that the *A. darlingi* human-biting rate is proportional to the amount of land altered by humans and inversely proportional to the amount of remaining forest. To this end, we performed ordered-logit regression, classifying *A. darlingi* as “low” (0–0.09/hr), “medium” (0.1–0.9/hr), or “high” (1.0–3.8/hr) (Statistical Software, 7.0 ed., Stata Corporation, College Station, TX). We first explored the effects of each landscape and human population density variable individually. We then combined the variables with the strongest effects to obtain a model with good predictive ability. Because the landscape variables are compositional in nature, multicollinearity posed a problem when combining variables such as grass/crop land with forest. This was dealt with by avoiding adding collinear variables in the model and then checking the variance inflation factor. Interaction terms were also examined and included when significant. Spatial autocorrelation was checked using the Durbin-Watson statistic and by running a one-way ANOVA of the model residuals grouped by site clusters. Finally, the model was cross-validated using five randomly selected subsets of data.

RESULTS

Anopheles prevalence and characteristics. A total of 15,325 anophelines were captured, 2,646 of which were adult *A. darlingi* (an average of 3.0 *A. darlingi* were captured per 6-hour collection night). Among the anophelines collected, *A. darlingi* was second in abundance after *A. triannulatus* (4.3 per 6-hour collection night). Other potential malaria vectors collected were *A. benarrochi*, *A. oswaldoi*, *A. nuneztovari*, and *A. rangeli*.^{12,18,21,23,27} Nonvectors included *A. mediopunctatus*, *A. mattogrossensis*, *A. punctimacula*, *A. peryassui*, *A. neomaculipalpus*, and *A. squamifemur*. In Table 1, the human-biting rates of each species collected are shown stratified by the amount of forest in a 1 × 1 km grid surrounding the collection sites. *A. darlingi* was captured in the greatest quantities at sites with little remaining forest (0–20%). The *A. darlingi* human-biting rate was one sixth to one third of this

rate in sites with intermediate amounts of forest (20–60%) and scarce in sites with more than 60% forest. None of the other *Anopheles* species exhibit such a marked preference for areas with little remaining forest. The human-biting rate of culicines collected is shown for reference. Culicines were vastly more abundant, and as a group, large differences in human-biting rates are not apparent across vegetation categories.

The *A. darlingi* biting rate on humans was 2.8 (95% confidence intervals [1.8, 3.8] per 6 hours per person) in the dry season (between September and November 2000) and declined to 0.9 (0.5, 1.2) during the rainy season (between February and April 2001). The biting rate on humans rose again after the rainy season between May and August 2001 to 4.9 (3.5, 6.3) (Figure 2). The peak biting time occurred between 9 and 10 PM.

Landscape features and human-biting rates. The average amounts of each landscape type in a 200 × 200 m, 2 × 2 km, and 7 × 7 km area surrounding the collection site are shown for the “low,” “medium,” and “high” categories of *A. darlingi* mean biting-rates in Table 2. The Kruskal-Wallis test was used for each grid size to test the null hypothesis that the “low,” “medium,” and “high” *A. darlingi* categories have equal population medians.²⁸ This table shows that the amounts of secondary growth, shrub, and grass/crop land increase for the “medium” and “high” categories compared with the “low” category, while the amount of forest is inversely related. The scale at which these forces operate does appear to be relevant, especially for the amount of bare surface and deep water. A similar table is subsequently shown (Table 3), comparing the mean distances from the collection site to the nearest edge of the various landscape categories for each *A. darlingi* category. Again, the Kruskal-Wallis test reveals that the medians for the distance to forest, secondary growth, shrub, and grass/crop land are significantly different across *A. darlingi* biting rate categories. Table 4 compares the mean number of human inhabitants in 200-m, 500-m, and 1-km radii across the human biting rate categories, the proportion of sites with one or more people within these radii, as

TABLE 1

Average human-biting rates and 95% confidence intervals for each *Anopheles* species by the amount of remaining forest in a 1 × 1 km grid around the collection site (as determined by remote sensing and GIS)

Species	Percent remaining forest		
	0–20% (19 sites)	20–60% (24 sites)	60–98% (13 sites)
Subgenus Nyssorhynchus			
<i>A. darlingi</i>	6.5 (4.9, 8.0)*	1.7 (1.0, 2.4)	0.0 (0.0, 0.1)
<i>A. triannulatus</i>	1.2 (0.6, 1.8)	5.0 (3.1, 6.8)	7.6 (5.3, 9.9)
<i>A. benarrochi</i>	2.5 (1.5, 3.5)	1.6 (0.1, 3.1)	1.4 (0.0, 3.9)
<i>A. oswaldoi</i>	1.0 (0.1, 1.8)	2.2 (1.5, 2.8)	1.4 (0.8, 2.0)
<i>A. rangeli</i>	0.5 (0.3, 0.7)	0.9 (0.5, 1.3)	0.0 (0.0, 0.0)
<i>A. nuneztovari</i>	0.3 (0.1, 0.5)	0.1 (0.1, 0.2)	0.1 (0.0, 0.1)
<i>A. (N) spp.†</i>	0.8 (0.5, 1.2)	0.9 (0.7, 1.2)	0.6 (0.4, 0.8)
Subgenus Anopheles			
<i>A. mediopunctatus</i>	0.4 (0.2, 0.5)	1.9 (1.3, 2.4)	1.1 (0.7, 1.6)
<i>A. mattogrossensis</i>	0.2 (0.1, 0.3)	0.4 (0.1, 0.8)	0.0 (0.0, 0.0)
<i>A. punctimacula</i>	0.0 (0.0, 0.1)	0.2 (0.0, 0.3)	0.2 (0.0, 0.5)
<i>A. peryassui</i>	0.0 (0.0, 0.1)	0.0 (0.0, 0.0)	0
<i>A. (A) spp.†</i>	0.1 (0.0, 0.1)	0.1 (0.1, 0.2)	0.1 (0.0, 0.2)
Total Anopheles	13.5 (11.1, 15.8)	28.4 (5.7, 51.0)	12.7 (9.1, 16.3)
Culicines	192.7 (171.3, 214.1)	258.2 (228.8, 287.6)	297.3 (243.3, 351.2)

* Mean human-biting rate per 6 hours per person; 15 or 16 collection nights per site during 1 year (total: 888 6-hour nights).

† (N) = s.g. *Nyssorhynchus*, (A) = s.g. *Anopheles*. Due to low numbers, *A. neomaculipalpus* (three specimens total) and *A. squamifemur* (subgenus *Lophopodomyia*; 13 specimens total) were omitted from the table.

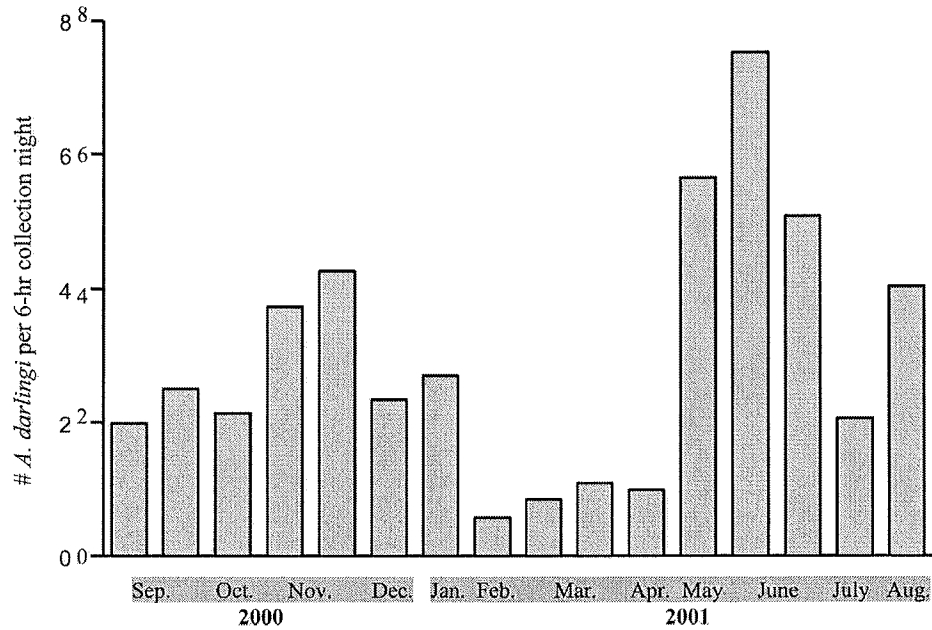


FIGURE 2. Average number of *A. darlingi* collected per person between 6 PM and midnight (all sites combined) by collection period. Collections took place once every 3 weeks (16 collections total).

well as the distance along the road to Iquitos. Although the median number of inhabitants are significantly different for the three *A. darlingi* categories, the highest numbers are seen in the “medium” category. However, the proportion of sites with at least one inhabitant is consistently higher for the “high” *A. darlingi* category. The last variable, the mean distance to Iquitos, is substantially different for each *A. darlingi* biting-rate category and shows that sites farther away from the city have lower biting rates than their proximal counterparts.

Sites with more than 70% forest and less than 10% grass/crop land in a 1-km² area (*N* = 10) had a mean human-biting rate of 0.03 *A. darlingi* per 6-hour collection night per person

[95% confidence intervals (0.01, 0.08)], whereas sites with less than 20% forest and more than 30% grass/crop land (*N* = 9) had a mean biting rate of 8.33 (7.86, 8.81). This amounts to a 278-fold higher biting rate in the grass/crop land sites compared with the relatively pristine sites.

The bodies of deep water represent large natural ponds or fish farms. The sites closer to these bodies of water were generally surrounded by more early- and late-stage deforestation (mean values: grass/crop land, 27%; shrub, 33%; secondary growth, 18%) than the sites lying farther away (grass/crop land, 10%; shrub, 17%; secondary growth, 16%). One or more inhabitants was more likely to be present in sites close to bodies of deep water (90%) than in sites distant from water

TABLE 2

Comparison of mean percentages of each landscape category (at the 0.2 × 0.2 km, 2 × 2 km, and 7 × 7 km grid levels) by *A. darlingi* human-biting-rate category (Kruskal-Wallis test)

Scale	<i>A. darlingi</i> biting rate/hr									P value		
	Low (0–0.09) <i>N</i> = 35			Medium (0.1–0.9) <i>N</i> = 12			High (1.0–3.8) <i>N</i> = 9					
	0.2 km	2 km	7 km	0.2 km	2 km	7 km	0.2 km	2 km	7 km	0.2 km	2 km	7 km
Varilla ^a	3.5	4.6	4.3	0.3	1.9	4.1	1.0	3.4	4.4	NS	*	NS
Forest ^b	48.0	53.9	58.0	17.2	31.9	43.9	9.4	23.3	32.2	***	****	****
Secondary growth ^c	14.4	15.9	15.0	14.9	22.6	20.7	12.7	21.3	21.9	NS	*	****
Shrub ^d	19.9	15.6	14.5	17.6	27.7	21.4	43.5	29.2	28.1	*	***	***
Grass/crop land ^e	12.8	9.5	7.6	47.5	15.1	9.1	31.7	20.4	11.8	***	***	**
Bare surface ^f	1.2	0.3	0.2	2.5	0.4	0.4	1.6	1.8	0.7	NS	NS	***
Shallow water ^g	0	0.1	0.1	0	0.2	0.1	0	0.2	0.5	NS	NS	NS
Deep water ^h	0.2	0.1	0.2	0	0.2	0.3	0	0.4	0.6	NS	NS	*

NS, not significant; **P* < 0.05, ***P* < 0.01, ****P* < 0.005, *****P* < 0.001.

^a Local term for forests with poor drainage, sandy soils, and a relatively sparse canopy.

^b Tall forest, dense canopy, with little evidence of human exploitation (small-scale exploitation invariably occurs in this region).

^c Tall, older secondary forest (forest that had been deforested approximately 15 years prior to the study) with a canopy height of 5–15 m.

^d Younger secondary growth (forest that had been deforested approximately 5 years prior to the study) with a height of 2–5 m.

^e Grass or low vegetation (< 2 m).

^f Compacted soil, asphalt, tin roofs.

^g Bodies of water that are at least 20 × 20 m (for satellite detection), deep, and with low amounts of sedimentation.

^h Bodies of water that are at least 20 × 20 m, shallow, and/or heavily sedimented (often lining the banks of deep water bodies).

TABLE 3

Comparison of mean distances from the collection site to the nearest edge of each landscape category by *A. darlingi* human-biting-rate category (Kruskal-Wallis test)

Distances to the nearest edge of (in meters)	<i>A. darlingi</i> biting rate/hr			P value
	Low (0.0-0.09) N = 35	Medium (0.1-0.9) N = 12	High (1.0-3.8) N = 9	
Varillal	178	300	225	NS
Forest	43	124	100	**
Secondary growth	62	67	67	NS
Shrub	164	62	17	*
Grass/crop land	211	76	40	*
Bare surface	841	733	357	*
Shallow water	2,256	1,306	1,040	NS
Deep water	1,549	710	746	NS

NS, not significant; *P < 0.05, **P < 0.01, ***P < 0.005, ****P < 0.001.

(49%). Conversely, the mean amount of forest was substantially less around the former group of sites (19%) than the latter (50%). The mean *A. darlingi* biting rate per 6 hours for sites closer to deep water was 4.8, and for farther sites it was 1.9.

Association between biting rates of *A. darlingi* and environmental factors. The variables giving rise to the ordered-logit model with the best fit were combined while avoiding problems of multicollinearity. The odds ratios are best understood as the odds of being in a higher category for every one unit change in the independent variable. These variables are 1) the percent grass/crop land in a 200 × 200 m grid (OR = 1.10 for every 1% increase, 95% CI [1.03, 1.17]), 2) the distance to the nearest edge of shrub (< 100 m, or ≥ 100 m) (not statistically significant), 3) the interaction term of these two variables (OR = 0.89 for every 1% increase in grass/crop land given that the distance to shrub < 100 m, 95% CI [0.81, 0.99]), 4) the percent deep water in a 7 × 7 km grid (OR = 186.69 for every 1% increase, 95% CI [1.98, 17,618.61]), 5) the distance to Iquitos along the road (OR = 0.71 for every 1 km, 95% CI [0.59, 0.86]), and 6) the presence of humans in a 500-m radius (OR = 395.45 present versus absent, 95% CI [5.79, 27,006.77]). The cutoff value for the “low” *A. darlingi* category is -3.36 (P[A. *darlingi* = “low”] = P[linear function of independent variables + u ≤ -3.36]), and 0.60 for the “medium” category (P[A. *darlingi* = “medium”] = P[-3.36 < linear function of independent variables + u ≤ 0.60], and P[A. *darlingi* = “high”] = P[linear function of independent variables + u > 0.60]). The pseudo-R² was 0.65, and the variance inflation factor assessing for multicollinearity was < 1.94 (> 5.0 is considered problematic).

Spatial autocorrelation was tested using negative binomial regression of the *A. darlingi* count per 6-hour collection and the model variables. The residuals plotted by distance along the road revealed a random pattern, with two outliers (the two villages closest to Iquitos). The one-way ANOVA (with residuals grouped by the distance along the road) was only significant for the two villages near Iquitos compared with the remaining sites. The Durbin-Watson statistic was 1.88 (critical values for 56 observations and six variables are dL = 1.41, dU = 1.77). However, excluding the variable “distance to Iquitos” resulted in a Durbin-Watson statistic of 1.12. We concluded therefore that there is no spatial autocorrelation given that we have adjusted for the distance to Iquitos.

Cross-validation of the model was then performed on five

randomly selected subsets of the data. The model correctly predicted 80–100% of the “low” *A. darlingi* sites, 33–50% of the “medium” *A. darlingi* sites, and 0–100% of the “high” sites. For the whole data set, the model correctly predicted 94% of the “low” sites, 64% of the “medium” sites, and 78% of the “high” sites (85% correctly predicted overall) (Table 5).

The percent remaining forest could not be incorporated into this model due to collinearity. Because it is very significantly associated with *A. darlingi* biting rates, it is modeled separately and shown in Figure 3 (adjusted for the effect of distance along the road). This graph shows the increasing probability that a site will fall into the “low” *A. darlingi* category with increasing forest, the decreasing probability that a site will be classified as a “high” *A. darlingi* site with increasing forest, and an initially increasing, then decreasing probability that a site will fall into the “medium” *A. darlingi* category.

DISCUSSION

Examination of the raw data as well as the multivariate analysis yields a consistent picture of higher *A. darlingi* human-biting rates in areas with more deforestation, even after controlling for the presence of humans. Our larval studies suggest that this association results from *A. darlingi*'s preference to breed in grass/crop land with secondary growth and shrub, leading to a corresponding rise in the adults of this species in the same area (unpublished). Though rarely systematized, *A. darlingi* breeding sites have been described as being partially shady, deep, and clean bodies of water, often associated with rivers and floating vegetation.^{13,29} In our study region, the rivers were probably beyond the flight range of *A. darlingi* (the site closest to a river was 2.5 km, with most sites situated approximately 8 km from the nearest river).^{19,30} Large ponds and fish farms surrounded by some vegetation (consisting of partially shady, deep, and clean bodies of water) were the most common *A. darlingi* breeding sites in this area. The low abundance of this vector in heavily forested areas, relative to the number of other mosquito species captured, suggests that *A. darlingi* is probably not breeding in forests. This was also supported by our larval studies.

The effect of each grid size was examined in the exploratory analysis. The importance of scale varied with landuse class. For forest, a positive association with *A. darlingi* biting rates was observed at each grid size. For bare surface and deep water, the association was most pronounced at the largest grid size (7 × 7 km). Grass/crop land, however, showed a stronger association at the smaller grid sizes.

One of the aims of this study was to separate the effect of deforestation from that of human presence. The initial design facilitated this by geographically matching deforested areas that were densely populated with ecologically similar areas that were sparsely populated. In the multivariate analysis, only the presence/absence of inhabitants in a 500-m radius around the collection site was significant. The effect of the number of inhabitants in a 500-m radius (population density) on *A. darlingi* biting rates was also examined but showed no linear trend. Thus, although the presence of humans was significant, the population density in excess of 1 person in a 500-m radius was not, and the effect of the ecological variables remained significant upon adjustment. Among these

TABLE 4

Comparison of mean number of human inhabitants, the proportion of sites with human presence, and distance from Iquitos, by *A. darlingi* human-biting-rate category (Kruskal-Wallis test)

	<i>A. darlingi</i> biting rate/hr			P value
	Low (0.0-0.09) N = 35	Medium (0.1-0.9) N = 12	High (1.0-3.8) N = 9	
No. human inhabitants: 200-m radius	1.9	37.8	18.5	****
Human presence: 200-m radius (0 = absent, 1 = one or more inhabitants)	0.2	0.5	0.8	***
No. human inhabitants: 500-m radius	9.9	111.5	40.3	***
Human presence: 500-m radius (0 = absent, 1 = one or more inhabitants)	0.5	0.8	1.0	**
No. human inhabitants: 1-km radius	45.2	189.5	76.3	*
Human presence: 1-km radius (0 = absent, 1 = one or more inhabitants)	0.8	0.8	1.0	NS
Distance from Iquitos (in km)	45.5	32.5	21.0	****

NS, not significant; *P < 0.05, **P < 0.01, ***P < 0.005, ****P < 0.001.

ecological variables, the amount of bare surface (composed of asphalt, compacted soil, tin roofing) would seem most likely to be a proxy for human activity and inhabitation. It was not a proxy for the latter, however, as shown by the low correlation (2%) between the amount of bare surface and the presence of people in a 500-m radius.

The seasonality and behavior of *A. darlingi* observed in our study exhibited some similarities as well as differences to that seen elsewhere in the Amazon. The significantly lower *A. darlingi* biting rate during the rainy season may have been due to a wash-out effect of breeding sites and lower temperatures, leading to decreased larval survival. After the rainy season, ample stable breeding sites were present and temperatures increased, creating conditions favorable to *A. darlingi*. This is similar to the situation described by Rozendaal in Suriname (1992).¹⁶ The late-night unimodal biting peak observed in our pilot study is consistent with other reports from the Amazon^{15,31,32} but is in contrast with the crepuscular biting pattern of *A. darlingi* observed in other regions of the Amazon, such as Rondonia, Costa Marques, and southeastern Amazonas, Brazil.^{19,33,34}

To understand the impact of deforestation on malaria transmission in humans, it would be useful to know the percentage of infective mosquitoes. This is a component of the entomological inoculation rate (EIR), which predicts the number of infective bites per person per unit of time. Because the human-biting rate is proportional to the EIR, it is highly likely that the higher *A. darlingi* biting rate in deforested areas signifies more malaria transmission when compared

with forested areas. The percent infective *A. darlingi* has been established in several studies in the Amazon and ranges between 0.5% and 2.1%.^{22,23} If the conservative 0.5% figure is used, the following EIRs would occur in our study site (considering deforestation in a 1 × 1 km grid): 38 infective bites per year would occur in an area with more than 35% grass/crop land (0.005 infective bites/*A. darlingi* bites × 365 nights/yr × 21.2 *A. darlingi* bites/night); 11 infective bites per year with 15–35% grass/crop land (0.005 infective bites/*A. darlingi* bites × 365 nights/yr × 5.8 *A. darlingi* bites/night); 8 infective bites per year for 2–15% grass/crop land (0.005 infective bites/*A. darlingi* bites × 365 nights/yr × 4.4 *A. darlingi* bites/night); and 0.1 infective bites per year for less than 2% grass/crop land (0.005 infective bites/*A. darlingi* bites × 365 nights/yr × 0.05 *A. darlingi* bites/night). This study has identified several landscape features that may be useful in predicting *A. darlingi* density and addressing the reduction of human-vector contact. The cross-validation of the multivariate ordered-logit regression achieved success in predicting sites with low *A. darlingi* biting rates but was less successful in predicting medium and high sites. Nonetheless, this could be a useful tool in predicting risk in areas of colonization and deforestation, such as the section of the Iquitos-Nauta road 65 km from Iquitos. These results may be generalizable to other areas of the Amazon where slash-and-burn agriculture predominates, but areas deforested for industrial agriculture (e.g., to grow soybeans), hydroelectric dams, or large-scale cattle ranches may experience different consequences.¹² In areas settled by subsistence farmers in Brazil, malaria epidemics often ensued

TABLE 5

Cross-validation of model for *A. darlingi* using five randomly selected data subsets

Trial no.	Percent correctly predicted as			Coefficients						Cut-off values		Pseudo-R ²
	Low	Medium	High	b1	b2	b3	b4	b5	b6	1	2	
1	6/6	1/2	3/3	-1.10	0.10	-0.10	5.12	-0.34	8.97	-0.46	3.46	0.66
2	4/5	1/2	1/3	-0.12	0.11	-0.15	0.22	-0.58	8.96	-10.46	-2.56	0.75
3	4/4	1/3	0/3	0.88	0.10	-0.13	6.96	-0.40	6.27	-4.09	1.11	0.70
4	8/8	0/0	2/2	0.25	0.09	-0.11	4.81	-0.33	5.84	-3.22	0.71	0.59
5	6/7	1/2	1/2	1.26	0.15	-0.19	6.96	-0.46	8.61	-3.49	1.73	0.71
Whole data set	32/34	7/11	7/9	0.20	0.09	-0.11	5.23	-0.34	5.98	-3.36	0.60	0.65

General form of the equation: S = b1(distance to shrub < or ≥ 100 m) + b2(% grass/crop land in 200 × 200 m) + b3(distance to shrub ≥ 100 m × % grass/crop land in 200 × 200 m) + b4(% deep water in 7 × 7 km) + b5(distance from Iquitos (km)) + b6(human inhabitants in 500 m < or ≥ 1).

Predicted probabilities: P(*A. darlingi* = "low") = P(S + u ≤ cut-off value 1), P(*A. darlingi* = "medium") = P(cut-off value 1 < S + u ≤ cut-off value 2), P(*A. darlingi* = "high") = P(cut-off value 2 < S + u).

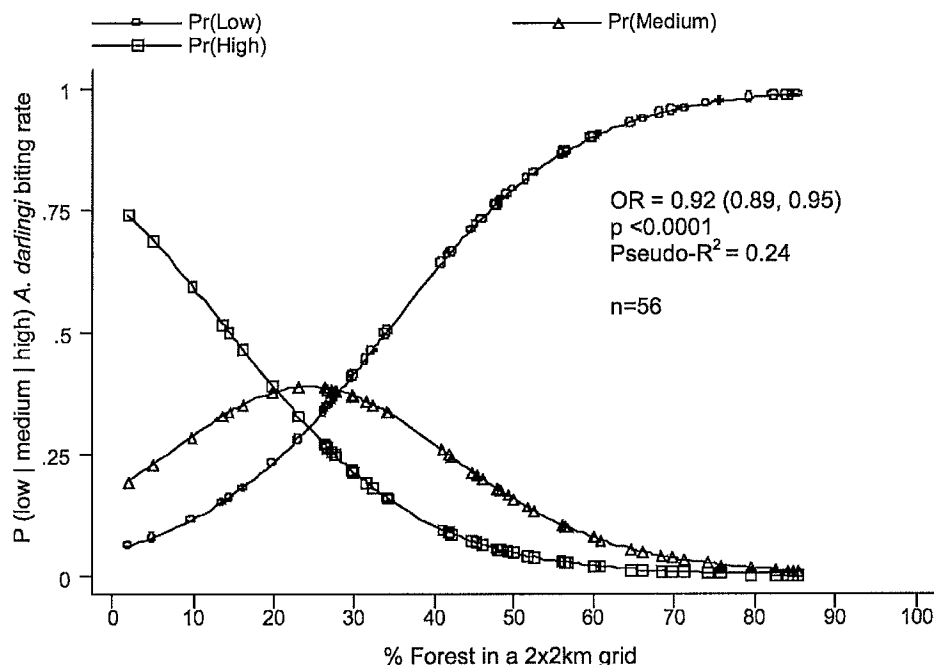


FIGURE 3. Ordered-logit regression showing the predicted probabilities of low, medium, or high biting rates by the percentage of forest. The predicted probability of a high biting rate decreases with increasing amounts of remaining forest, whereas the probability of a low biting rate increases proportionately with the amount of forest. The odds ratio of a high *A. darlingi* biting rate with every 1% increase in the % forest, P value, and pseudo- R^2 are shown for the ordered-logit regression.

as well. Unlike the scenario presented in this study, however, where epidemic malaria arose 10 to 15 years after the most intensive period of deforestation, in Rondonia, Brazil, new settlements (< 5 years) had significantly higher rates of malaria than older, established communities.³⁵ Control measures that reduce the amount of deforestation may be beneficial not only for management of malaria but also for the preservation of biodiversity and the reduction of erosion. Such control measures could include more sustainable methods of agriculture as well as human settlement guided by sound land use and conservation policy preventing widespread deforestation after road construction.

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REFERENCES

1. Gratz NG, 1999. Emerging and resurging vector-borne diseases. *Ann Rev Entomol* 55: 51-75.
2. Molyneaux DH, 2003. Common themes in changing vector-borne disease scenarios. *Trans R Soc Trop Med Hyg* 97: 129-132.
3. Walsh JF, Molyneaux DH, Birley MH, 1993. Deforestation: effects on vector-borne disease. *Parasitology* 106: S55-S75.
4. Kalliola R, Flores-Paitan S, 1998. Geocologia y desarrollo amazonico: estudio integrado en la zona de Iquitos, Peru. *Annales Universitatis Turkuensis Ser A II*. Turku, Finland: Turku University, 544.
5. Singer BH, de Castro MC, 2001. Agricultural colonization and

- malaria on the Amazon frontier. *Ann N Y Acad Sci* 954: 184–222.
6. Serra-Vega J, 1990. Andean settlers rush for Amazonia. *Earthwatch* 39: 7–9.
 7. Fearnside PM, 1993. Deforestation in Brazilian Amazonia—the effect of population and land-tenure. *Ambio* 22: 537–545.
 8. Flores-Paitan S, Gomez-Romero E, Kalliola R, 1998. Características generales de la zona de Iquitos. Kalliola R, Flores-Paitan S, eds. *Geoecologia y desarrollo Amazonico*. Sulkava: Finnreklama Oy, 17–31.
 9. Gomez-Romero E, Tamariz-Ortiz T, 1998. Uso de la tierra y patrones de deforestacion en la zona de Iquitos. Kalliola R, Flores-Paitan S, eds. *Geoecologia y Desarrollo Amazonico*. Sulkava: Finnreklama Oy.
 10. Povia MM, Conn JE, Schlichting CD, Amaral JCOF, Segura MNO, Da Silva ANM, Dos Santos CCB, Lacerda RNL, De Souza RTL, Galiza D, Santa Rosa EP, Wirtz RA, 2003. Malaria Vectors, epidemiology, and the re-emergence of *Anopheles darlingi* in Belm, Para, Brazil. *J Med Entomol* 40: 379–386.
 11. Downs WG, Pittendrigh CS, 1949. Bromeliad malaria in Trinidad, British West Indies. *Am J Trop Med Hyg* 26: 47–66.
 12. Tadei WP, Thatcher BD, Santos JMH, Scarpassa VM, Rodrigues JB, Rafael MS, 1998. Ecologic observations on anopheline vectors of malaria in the Brazilian Amazon. *Am J Trop Med Hyg* 59: 325–335.
 13. Consoli RAGB, Lourenço-de-Oliveira R, 1994. *Principais mosquitos de importancia sanitaria no Brasil*. Rio de Janeiro: Fiocruz.
 14. Horsfall WR, 1955. *Mosquitoes: Their Bionomics and Relation to Disease*. New York: The Ronal Press.
 15. Hudson JE, 1984. *Anopheles darlingi* Root (Diptera: Culicidae) in the Suriname rain forest. *Bull Entomol Res* 74: 129–142.
 16. Rozendaal JA, 1992. Relations between *Anopheles darlingi* breeding habitats, rainfall, river level and malaria transmission rates in the rain forest of Suriname. *Med Vet Entomol* 6: 16–22.
 17. Need JT, Rogers EJ, Phillips IA, Falcon R, Fernandez R, Carbajal F, Quintana J, 1993. Mosquitoes (Diptera: Culicidae) captured in the Iquitos area of Peru. *J Med Entomol* 30: 635–638.
 18. Calderon G, Fernandez R, Valle J, 1995. Especies de la fauna anofelina, su distribucion y algunas consideraciones sobre su abundancia e infectividad en el Peru. *Rev Per Entomol* 8: 5–23.
 19. Charlwood JD, Alecrim WA, 1989. Capture-recapture studies with the South American malaria vector *Anopheles darlingi*, Root. *Ann Trop Med Parasitol* 83: 569–576.
 20. Mather PM, 1999. *Computer Processing of Remotely-Sensed Images: An Introduction*. West Sussex: John Wiley & Sons.
 21. Rubio-Palis Y, 1994. Variation of the vectorial capacity of some anophelines in western Venezuela. *Am J Trop Med Hyg* 50: 420–424.
 22. Tadei WP, Dutary Thatcher B, 2000. Malaria vectors in the Brazilian Amazon: *Anopheles* of the subgenus *Nyssorhynchus*. *Rev Inst Med Trop S Paulo* 42: 87–94.
 23. Oliveira-Ferreira J, Lourenco-de-Oliveira R, Teva A, Deane LM, Daniel-Ribeiro CT, 1990. Natural malaria infections in Anophelines in Rondonia state, Brazilian Amazon. *Am J Trop Med Hyg* 43: 6–10.
 24. Charlwood JD, 1996. Biological variation in *Anopheles darlingi* Root. *Memorias do Instituto Oswaldo Cruz* 91: 391–398.
 25. Klein T, Lima JBP, Toda Tang A, 1991. Biting behavior of *Anopheles* mosquitoes in Costa Marques, Rondonia, Brazil. *Rev Soc Bras Med Trop* 24: 13–20.
 26. Oliveira-Ferreira J, Lourenco-de-Oliveira R, Deane LM, Daniel-Ribeiro CT, 1992. Feeding preference of *Anopheles darlingi* in malaria endemic areas of Rondonia state—Northwestern Brazil. *Mem Inst Oswaldo Cruz* 87: 601–602.
 27. Klein T, Lima JBP, Tada MS, Miller R, 1991. Comparative susceptibility of Anopheline mosquitoes in Rondonia, Brazil to infection by *Plasmodium vivax*. *Am J Trop Med Hyg* 45: 463–470.
 28. Hamilton LC, 1998. *Statistics with Stata 5*. Pacific Grove: Duxbury Press.
 29. Manguin S, Roberts DR, Andre RG, Rejmankova E, Hakre S, 1996. Characterization of *Anopheles darlingi* (Diptera: Culicidae) larval habitats in Belize, Central America. *J Med Entomol* 33: 63–69.
 30. Deane LM, Causey OR, Deane MP, 1948. Notas sobre a distribucão e a biologia dos anofelinos das Regioes Nordeste e Amazonica do Brasil. *Revista do Serv. Esp Saude Publica* 1: 827–966.
 31. Charlwood JD, 1980. Observations on the bionomics of *Anopheles darlingi* Root (Diptera: Culicidae) from Amazonas, Brazil. *Bull Entomol Res* 70: 685–692.
 32. Rozendaal JA, 1989. Biting and resting behaviour of *Anopheles darlingi* in the Suriname rainforest. *J Am Mosq Control Assoc* 5: 351–358.
 33. Roberts RD, Alecrim WD, Tavares AM, Radke MG, 1987. The house frequenting, host seeking and resting behavior of *Anopheles darlingi* in the southeastern Amazonas, Brazil. *J Am Mosq Control Assoc* 3: 433–441.
 34. Rosa-Freitas MG, Broomfield G, Priestman A, Milligan PJ, Momen H, Molyneux DH, 1992. Cuticular hydrocarbons, isoenzymes and behavior of three populations of *Anopheles darlingi* from Brazil. *J Am Mosq Control Assoc* 4: 357–366.
 35. McGreevy PB, Dietze R, Prata A, Hembree SC, 1989. Effects of immigration on the prevalence of malaria in rural-areas of the Amazon Basin of Brazil. *Mem Inst Oswaldo Cruz* 84: 485–491.