

## Determinants of *Anopheles* Seasonal Distribution Patterns Across a Forest to Periurban Gradient near Iquitos, Peru

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**Abstract.** As part of a field ecology study of arbovirus and malaria activity in the Amazon Basin, Loreto Department, Peru, we collected mosquitoes landing on humans at a forest site and inside and outside of residences and military barracks at periurban, rural, and village sites. We collected 11 *Anopheles* spp. from these four sites. *An. darlingi*, the principal malaria vector in the region, accounted for 98.7% of all *Anopheles* spp. collected at Puerto Almendra. Peaks in landing activity occurred during the December and April collection periods. However, the percent of sporozoite-positive *Anopheles* spp. was highest 1–2 months later, when landing activity decreased to approximately 10% of the peak activity periods. At all sites, peak landing activity occurred about 2 hours after sunset. These data provide a better understanding of the taxonomy, population density, and seasonal and habitat distribution of potential malaria vectors within the Amazon Basin region.

### INTRODUCTION

Malaria is on the increase in South America, reaching epidemic proportions in many countries and reemerging as a significant public health problem in others.<sup>1,2</sup> For example, the incidence of malaria in the Department of Loreto, Peru, increased sharply beginning in the early 1990s.<sup>3,4</sup> Concomitant with this increase in the 1990s was a change in the predominant causative agent of malaria from *Plasmodium vivax* to *P. falciparum* and the appearance of *Anopheles darlingi* Root as the primary vector, which was associated with these changes and increases of malaria in the Department of Loreto.<sup>3,5–7</sup> Although reasons for the changes in malaria transmission have not been clearly established, inadequate healthcare services, delayed diagnostics, poor housing, climate events (i.e., El Niño), inadequate vector control programs, banned use of dichlorodiphenyltrichloroethane (DDT), habitat modifications because of agriculture and deforestation, and reintroduction, reemergence, or expansion of *An. darlingi* all likely contributed.<sup>1,3,6,8,9</sup>

Because of its association with malaria throughout South America,<sup>10</sup> the bionomics of *An. darlingi* has been well-studied throughout much of the Amazon Basin. These studies, reviewed by Charlwood,<sup>11</sup> show that *An. darlingi* is exophilic and/or endophilic throughout its range. To examine the roles of *An. darlingi* and other *Anopheles* spp. in the resurgence of malaria in the vicinity of Iquitos, Peru, we evaluated the seasonal and daily human landing activity, exophilic and endophilic behavior, and seasonal *Plasmodium* spp. infection rates for *Anopheles* spp. collected at a Peruvian military base (periurban), two rural single dwelling residences, Puerto Almendra (rural village), and an adjacent forest site about 300 m from the village. The results reported here expand on the preliminary findings of the work by Turell and others.<sup>12</sup>

### MATERIALS AND METHODS

**Study site.** Mosquitoes were collected at several sites near Iquitos (approximate population is 350,000), Loreto

Department, in the Amazon Basin of northeastern Peru, approximately 125 m above sea level and bordered by the Amazon, Itaya, and Nanay Rivers (3°51'S, 73°13'W). The study sites are described in detail in the work by Jones and others,<sup>13</sup> and in addition, they include a periurban site (Fort Vargas Guerra, a Peruvian military training base partially surrounded by unmanaged lands and individual houses) in the city of Iquitos, Puerto Almendra (a forested rural village approximately 20 km west-southwest of Iquitos), a dense forest site about 0.3 km from Puerto Almendra, and two single dwelling residences: Casa de Sabino (located on the Nauta highway near the intersection of the dirt road in Quistococha that goes to Llanchama) and Casa de Juan (located on the road to Llanchama just under 1 km from the Nauta Highway and about 4.5 km from Puerto Almendra).

**Mosquito collections.** A total of nine adult mosquito collections were made from October of 1996 to September of 1997. Each collection consisted of four to six 24-hour collections at 12-hour intervals over 10–16 days. Human collectors, each wearing a hooded screened jacket to prevent biting on the upper parts of the body, conducted hourly human landing collections for 40 minutes starting on the hour followed by a 20-minute rest break from 0600 to 1800 hours (daytime collection) and from 1800 to 0600 hours (nighttime collection); they used oral aspirators under a human use protocol approved by the Naval Medical Research Center's and the US Army Medical Research Institute of Infectious Diseases' Institutional Review Boards in compliance with all applicable Federal regulations governing the protection of human subjects. Collectors were positioned both inside and approximately 5 m outside each resident-occupied house located adjacent to young forests and at ground level and 10 m in the forest canopy (forest site).

Mosquitoes were placed in humidified coolers at the end of each 12-hour collection period and transported to a central laboratory in Iquitos where they were identified according to the works by Lane,<sup>14</sup> Faran,<sup>15</sup> Faran and Linthicum,<sup>16</sup> and Wilkerson and Sallum.<sup>17</sup> A list of mosquito species identified from the collections is provided in the work by Pecor and others,<sup>18</sup> and preliminary mosquito bionomic findings are described in the works by Jones and others<sup>13</sup> and Turell and others.<sup>12</sup> Culicine mosquitoes were pooled (25–40 specimens)

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according to species and collection period, placed in sterile 1.5-mL cryovials, and maintained on dry ice or at  $-70^{\circ}\text{C}$  until assayed for the presence of viruses by plaque assay on Vero cell monolayers.<sup>19</sup> *Anopheles* spp. were similarly pooled (1–10 specimens) according to species and collection period, placed in 1.5-mL cryovials, and assayed by enzyme-linked immunosorbent assay (ELISA) for *P. falciparum*, *P. vivax*-210, and *P. vivax*-247 circumsporozoite antigen.<sup>20–22</sup> Voucher specimens were deposited in the Walter Reed Biosystematics Unit, Smithsonian Institution, Washington, DC, where field mosquito identifications were confirmed.<sup>18</sup>

**Animal hosts.** Domestic or peridomestic animals were not observed at Fort Vargas Guerra. However, a large fishpond adjacent to the medical clinic was home to water fowl. Dogs and chickens were the most common peridomestic animals observed at rural residences and the village of Puerto Almendra. Additionally, feral animals (i.e., squirrel monkeys [*Saimiri* spp.], wooley monkeys [*Lagothrix* spp.], bandicoots [*Nassua* spp.], and parrots) were kept as pets at some of the residences in Puerto Almendra. Grasslands with free-ranging water buffalo, cattle, and horses were located approximately 1 km from the forest and village collection sites. Numerous birds, including parrots, were observed at the forest study site near Puerto Almendra. The local population included hunters and gatherers who had virtually hunted much of the wild game, including rodents. Low numbers of animals were reported for forest areas surrounding Puerto Almendra, included rodents (*Proechimys* spp., *Oryzomys* spp., and *Neacomys* spp.), marsupials (*Philander* spp., *Metachirus* spp., and *Marmosops* spp.), and sloths (*Choloepus hoffmanni* and *Bradypus* spp.)

**Temperature and rainfall.** Rainfall data for Iquitos were provided by the Universidad Nacional de la Amazonía Peruana, and river level measurements for the Amazon River at Iquitos were provided by the Servicio de Hidrografía y Navegación de la Amazonia de la Marina de Guerra del Peru, Iquitos, Peru. The level of the Amazon River in Iquitos varies by as much as 10 m annually from 108 to 118 m above sea level, and it may affect mosquito populations.<sup>3</sup>

**Statistical analysis.** The total numbers of *An. darlingi* collected were correlated with the accumulated values of rainfall 15 days before the collection date. The distributions of numbers of mosquitoes and rainfall were tested to normality by the Kolmogorov–Smirnov test and the Spearman coefficient

( $\rho$ ;  $\alpha < 0.5$ ), and they were used in the analysis for correlations between relative numbers of mosquitoes and rainfall. The association between mosquito abundance (mean mosquitoes/collection per 24 hours either  $< 5$  or  $\geq 5$ ) and concurrent river levels (either  $< 112$  or  $\geq 112$  m above sea level) were examined using Fisher's exact test. The entomological inoculation rate (EIR) was calculated by multiplying the human landing rate times the infection rate and then adjusting for a monthly rate by multiplying by 30.

## RESULTS

**Anopheline composition and distribution.** We collected 10,748 *Anopheles* mosquitoes representing 11 species from three subgenera as they landed on the collectors (Table 1). Overall, three species, *An. darlingi*, *An. benarrochi* Gabaldon, Cova Garcia, and Lopez, and *An. nuneztovari* Gabaldon, accounted for 94.7% of all anopheline mosquitoes collected (Table 1). *An. darlingi* was the predominant *Anopheles* spp. collected at the rural residences (66.6%) and the village of Puerto Almendra (98.7%). Despite total mosquito collection rates being 10-fold higher at the forest site than the nearby village of Puerto Almendra,<sup>12</sup> 300-fold more *An. darlingi* was collected at Puerto Almendra. Although *An. benarrochi*, *An. nuneztovari*, and *An. oswaldoi* (Peryassu) were rarely collected at Puerto Almendra, these species were routinely collected at rural residences that were bordered by young forests. *An. triannulatus* (Neiva and Pinto) was collected similarly at both Puerto Almendra and the forest site. Although not commonly collected at the other sites, *An. kompi* Edwards and members of the *An. forattinii* Wilkerson and Sallum and *An. mediopunctatus* (Lutz) complexes (perhaps *An. costai* Fonseca and Ramos and a species related to *An. forattinii*; Wilkerson R, personal communication) were the most commonly collected *Anopheles* spp. at the forested site. Only a few *Anopheles* mosquitoes were collected at Fort Vargas Guerra ( $< 10\%$  and  $< 1\%$  compared with the forest site and the rural or village sites, respectively). However, more *An. mattogrossensis* Lutz and Neiva were collected at Fort Vargas Guerra than at any of the other sites.

**Comparison of mosquitoes collected inside and outside of houses.** Paired human collectors collected mosquitoes inside and outside houses at Puerto Almendra for 84 trap

TABLE 1  
*Anopheles* spp. collected at human landing collections in the vicinity of Iquitos, Peru, from October of 1996 to September of 1997

Species	Number (%) of adult females collected by site*				
	Periurban area†	Rural area	Forest village‡	Forest	Totals
<i>Anopheles (Nys.) darlingi</i>	11 (38)	2,671 (67)	6,225 (99)	20 (5)	8,927 (83)
<i>Anopheles (Nys.) benarrochi</i>	0 (0)	795 (20)	7 (< 1)	2 (< 1)	804 (8)
<i>Anopheles (Nys.) nuneztovari</i>	0 (0)	432 (11)	13 (< 1)	9 (2)	454 (4)
<i>Anopheles (Ano.) forattinii</i> §	0 (0)	7 (< 1)	32 (< 1)	150 (40)	189 (2)
<i>Anopheles (Ste.) kompi</i>	0 (0)	5 (< 1)	1 (< 1)	163 (43)	269 (2)
<i>Anopheles (Nys.) oswaldoi</i>	0 (0)	66 (2)	7 (< 1)	18 (5)	91 (< 1)
<i>Anopheles (Nys.) triannulatus</i>	7 (24)	25 (< 1)	7 (< 1)	23 (6)	62 (< 1)
<i>Anopheles (Ano.) mattogrossensis</i>	11 (38)	4 (< 1)	5 (< 1)	3 (< 1)	23 (< 1)
<i>Anopheles (Ano.) shannoni</i>	0 (0)	2 (< 1)	7 (< 1)	9 (2)	18 (< 1)
<i>Anopheles (Ano.) peryassui</i>	0 (0)	4 (< 1)	6 (< 1)	0 (0)	10 (< 1)
<i>Anopheles (Nys.) rangeli</i>	0 (0)	1 (< 1)	0 (0)	0 (0)	1 (< 1)
Total	29	4,012	6,310	397	10,748

\*Sum of 168 24-hour collections (40 minutes/hour; indoors and outdoors at all sites except the forest site, where collections were made at ground level and in the canopy [10 m] at each site).

†Fort Vargas Guerra.

‡Village of Puerto Almendra located about 300 m from the forest collection site.

§Consists of two species: *Anopheles forattinii* and a species related to *An. mediopunctatus*.

TABLE 2

Association between mosquito landing rates and sporozoite infection rates in *An. darlingi* collected by human volunteers in the vicinity of Iquitos, Peru, from October of 1996 to September of 1997

Collection period	Landing rate*	Infection rate†	Number tested (number infected)	EIR per month‡
October 23 to November 4, 1996	61	0.3	906 (3)	5.5
December 3–13, 1996	80	0.5	1,161 (6)	12.0
January 14–28, 1997	20	1.1	471 (5)	6.7
February 26 to March 9, 1997	15	0	95 (0)	0
April 7–14, 1997	165	0.1	3,024 (3)	5.0
May 20 to June 4, 1997	83	0	885 (0)	0
July 1–16, 1997	11	3.1	129 (4)	10.2
August 12–25, 1997	5	0	0 (0)	0
September 17 to October 1, 1997	3	0	48 (0)	0

\*Number of *An. darlingi* captured per 24-hour period per person.

†Number of *An. darlingi* containing *P. falciparum* sporozoites per 100 tested.

‡Entomologic inoculation rates = landing rate  $\times$  infection rate/100  $\times$  30.

days to determine which mosquito species readily entered dwellings. Over a 24-hour period, similar numbers of *An. darlingi* were collected inside (3,969) and outside (3,955) of houses. Although relatively few *An. darlingi* (342/7,924) were collected during the daytime (0600–1800 hours), significantly more were collected inside (62.3%; binomial test,  $P < 0.001$ ) than outside of houses.

**Time of day of *An. darlingi* activity.** A total of 96.2% (8,585/8,927) *An. darlingi* was collected between 1800 and 0600 hours, whereas only 3.8% (342/8,927) were collected during the daytime (0600–1800 hours). Although 73% of *An. darlingi* were collected during the evening (1800–2400 hours), only 27% were collected after midnight (2400–0600 hours).

**Biting and infection rates among *An. darlingi*.** We detected *P. falciparum* sporozoites (circumsporozoite protein) in 21/6,719 *An. darlingi* by ELISA (Table 2). At Puerto Almendra, *An. darlingi* populations fluctuated throughout the seasons, with peak numbers collected during December of 1996 and April of 1997. In contrast, the highest sporozoite infection rates were detected approximately 1–2 months later (January and July of 1997) (Figure 1). In addition to the detection of *P. falciparum* in 21 *An. darlingi*, the variant Pv210 of *P. vivax* was detected from a single unidentified *Anopheles* (*Nyssorhynchus*) spp.

**Landing rate and river level.** The numbers of *An. darlingi* collected at Puerto Almendra coincided with the river level,

with the increased numbers collected in December and April associated with the rising levels of the Amazon River in addition to other associated rivers and tributary systems (Figure 2).

## DISCUSSION

*An. darlingi* was the most frequently collected *Anopheles* spp. in human landing collections where people resided (98.7%, 66.6%, and 37.9% at Puerto Almendra, two rural residences, and Fort Vargas Guerra, respectively). However, as recently as 1994, this species was not reported from this region.<sup>5,6</sup> The rapid increase in the prevalence of this species is believed to be one of the causes for recent increases in malaria cases in this region.<sup>3,9</sup> Although *An. darlingi* readily bites humans and was collected in large numbers at human landing collections, it is not efficiently collected by miniature light traps, and population estimates based on light trap data may be misleading. For example, in an earlier study in Puerto Almendra, > 24-fold more *An. darlingi* were collected at human landing collections than at dry ice-baited miniature light trap collections conducted concurrently about 10 m apart.<sup>12</sup>

The principle larval habitat for *An. darlingi* is among emergent and stationary floating vegetation along streams and river margins in forested areas.<sup>23,24</sup> Dispersal of *An. darlingi*

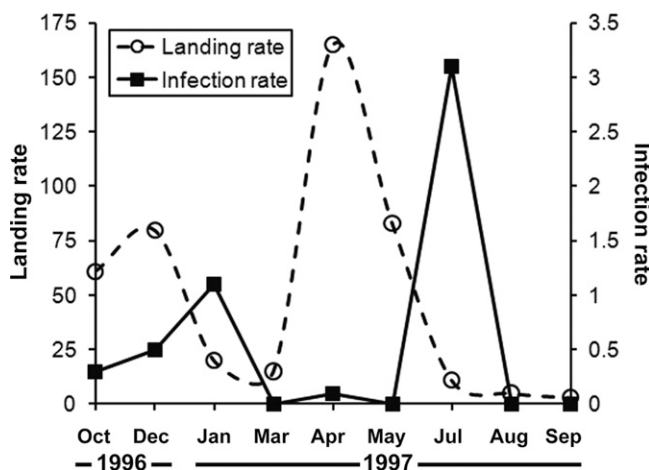


FIGURE 1. Number of *An. darlingi* collected per 24-hour period per collector and *P. falciparum* sporozoite infection rates (number positive/100 tested) for *An. darlingi* from Puerto Almendra, Peru, from October of 1996 to September of 1997.

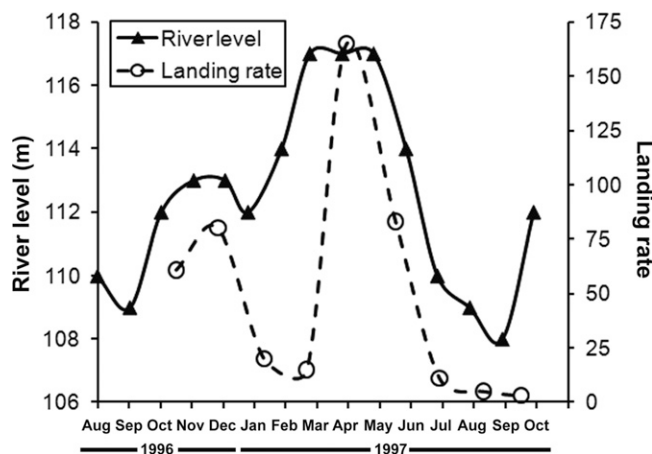


FIGURE 2. Relationship between river level (m above sea level) and landing rates (number of *An. darlingi* captured per 24-hour period per collector) at Puerto Almendra, Peru, from October of 1996 to September of 1997.

TABLE 3

Percentage (number) of *An. darlingi* captured by human collectors indoors and outdoors at various locations in the vicinity of Iquitos, Peru, from October of 1996 to September of 1997

Location	Daytime	Nighttime	Total
Casa de Juan	63 (197)*	56 (1,608)	56.6 (1,805)
Casa de Sabino	43 (7)	48 (245)	47.6 (252)
Puerto Almendra	62 (138)	48 (5,729)	48.2 (5,867)
Total	62 (342)	50 (7,582)	50.1 (7,924)

\*Percentage of *An. darlingi* captured indoors (number captured) at landing collections.

occurs as floating stationary vegetation breaks free from flooded areas, tributaries, and streams and flows downstream. Although larvae of *An. darlingi* are associated with forest streams and rivers, adults seek open (often deforested and disturbed areas) adjacent to forested sites where people reside for bloodmeals.<sup>9,25</sup> This preference is consistent with our results, because human landing collections conducted concurrently at Puerto Almendra (an open area village surrounded by a forest) and a forest site (located about 300 m from the village collection site) resulted in > 300 times as many *An. darlingi* collected in the village, although > 10 times more mosquitoes (culicines and anophelines) were collected at the forest site.<sup>12</sup> Therefore, it may not be the disturbance of the natural environment that allows for the increase in *An. darlingi*, but rather, this disturbance allows for an increase in the efficiency in capturing this species. Similarly, we collected more *An. darlingi* at Puerto Almendra (< 0.5 km from the Nanay River) and where there were large numbers of people than from either of the two rural residences located in a disturbed area about 3–4 km from the nearest river. This finding is consistent with the work of Roberts and others<sup>26</sup> and may explain the relationship between river levels, human populations, and *An. darlingi* populations (Figure 2).<sup>27</sup>

We found that 96.2% of landing activity took place during the hours of darkness (1800–0600 hours), whereas only 3.8% occurred during the daytime (0600–1800 hours). At all sites sampled, peak landing activity occurred about 2 hours after sunset, with 73% of all *An. darlingi* collected between 1800 and 2400 hours. This finding is consistent with the observations of Roberts and others<sup>28</sup> in Brazil and the data previously reported in the work by Turell and others<sup>12</sup> for the Peruvian Amazon Basin. Nearly all of the daytime human landing activity for *An. darlingi* occurred when seasonal populations peaked during April. Although similar numbers of *An. darlingi* were collected indoors and outdoors during the hours of darkness, significantly more (62.3%) were collected indoors than outdoors during the daytime collections (Table 3). This result may be because of the relative darkness inside houses when resting mosquitoes were disturbed as people moved about and indicates that some of the *An. darlingi* were using households as daytime resting locations. This finding has implication for indoor residual spraying (IRS). Although only a relative small percentage of the *An. darlingi* seemed to be resting during the daylight hours in these houses, IRS might actually be effective in killing those individuals that rested on the wall, even if only briefly, during the evening hours.

*An. darlingi* was the only species observed with *P. falciparum* sporozoites, confirming it as a primary vector of malaria in this region. Peak sporozoite infection rates occurred 1–2 months after peak populations of *An. darlingi*. This finding may be because of increased longevity of adult mosquitoes after the

rainy season (because of lower temperatures) and increased numbers of asymptomatic and symptomatic malaria cases during the rainy season that precedes the receding river levels (Figure 2).<sup>3</sup> The lower temperatures observed during the dry season may have little effect on the extrinsic development of *Plasmodium* spp. as temperatures fall within the optimal range of development.<sup>3</sup> However, a more important measure of risk of infection with malaria is the EIR, which accounts for both the number of mosquitoes attempting to bite and the infection rate in these mosquitoes.<sup>29</sup> In our study, the EIR remained elevated, even after mosquito populations began to fall, indicating the need for continued mosquito control efforts, even as natural populations were declining.

In summary, the reintroduction or expansion of *An. darlingi* in urban and rural areas of growth and increased human populations combined with inadequate healthcare services, delayed diagnostics, poor housing (unscreened doors and windows), climate events (i.e., El Niño), inadequate vector control programs, discontinuance of the use of DDT, and habitat modifications (i.e., logging, agriculture, and urban expansion) have greatly contributed to the rapid increase and expansion of malaria in many parts of the Amazon Basin, including Iquitos and the surrounding area.<sup>1,3,6,8,9</sup>

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