Seasonal Distribution, Biology, and Human Attraction Patterns of Mosquitoes (Diptera: Culicidae) in a Rural Village and Adjacent Forested Site Near Iquitos, Peru

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ABSTRACT This study was conducted as part of a field-ecology study of arboviral and malarial activity in the Amazon Basin, Loreto Department, Peru, to determine the relative abundance, species diversity, and seasonal and vertical distributions of potential mosquito vectors. Mosquitoes were captured either by volunteers using mouth aspirators while mosquitoes attempted to land on the collectors or in dry ice–baited ABC light traps. *Anopheles darlingi*, the principal malaria vector in the region, was the most commonly captured anopheline mosquito in Puerto Almendra village (99%) while landing on humans, with a mean of 37.1 mosquitoes captured per 24-h period, representing nearly one half of all mosquitoes collected. *An. darlingi* human landing activity began shortly after sunset, peaked at 2000–2100 hours, and declined gradually until sunrise. This species readily entered houses, because 51% of the *An. darlingi* captured by paired collectors, stationed inside and outside houses, were captured indoors. Human landing collections provided a more accurate estimate of human attraction of *An. darlingi*, capturing 30 times as many as co-located dry ice–baited ABC light traps. In contrast, eight times as many *Culex* (*Melanoconion*) species, including known arbovirus vectors, were captured in light traps as by co-located human collectors. Despite being located within 300 m of the village collection site, only a few *Anopheles* species were captured at the forest collection site, including only 0.1 *An darlingi*/24 h, thus indicating that *An. darlingi* activity was directly associated with the rural village. These data provide a better understanding of the taxonomy, population density, and seasonal distribution of potential mosquito vectors of disease within the Amazon Basin region and allow for the development of appropriate vector and disease prevention strategies that target vector populations.

KEY WORDS *Anopheles*, bionomics, mosquito ecology, Amazon Basin, Peru

Malaria and other arthropod-vectored diseases are on the increase in South America, reaching epidemic proportions in many countries, while reemerging as a major health problem in others (Roberts et al. 1997, Watts et al. 1998, Aramburu Guarda et al. 1999, Tesh et al. 1999, Schoeler et al. 2003, Flores-Mendoza et al. 2004). Although a few of the potential vectors have been identified, vector distribution, epidemiology of associated diseases, and risk factors associated with the transmission of these pathogens remain poorly understood throughout most of the Amazon Basin. Human migration, establishment of rural villages and urban communities, dispersal of single family dwellings on farmlands, resultant habitat modifications caused by agriculture and forestry activities, and introduction of domestic animals increases exposure to vector populations and the pathogens that they carry. An understanding of the biology and ecology of potential mosquito vectors is crucial for disease-threat analysis and for the development and implementation of vector-disease control strategies.

Because of its association with malaria throughout South America, the bionomics of *Anopheles darlingi* Root has been studied throughout parts of Central and

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South America. These studies have been reviewed by Charlwood (1996), who reported that this species can be either exophilic or endophilic and either endophagic or exophagic throughout its range. To examine the potential role of *An. darlingi* in the resurgence of malaria and of other culicine species as potential arbovirus vectors in the region around Iquitos, Peru, we evaluated the bionomics of mosquito species in Puerto Almendra, a rural village, and an adjacent densely forested site ≈300 m from the village center. We determined seasonal activity, daily biting activity, relative attractiveness of human collectors and dry ice–baited light traps, indoor versus outdoor prevalence, as well as comparing mosquito species captured in the village with those species captured in the nearby forest.

This study was part of a vector ecology/arthropod-borne pathogen research program designed to evaluate the threat that mosquito-borne pathogens pose to human and animal health in this region and is the most comprehensive vector bionomics study conducted in the Amazon Basin region of Peru to date. The Puerto Almendra area was selected because human cases of dengue, malaria, Mayaro, Oropouche, Venezuelan equine encephalomyelitis, and large numbers of fevers of unknown origin were previously reported in and around nearby Iquitos (Phillips et al. 1992; Hayes et al. 1996; Watts et al. 1997, 1998; Tesh et al. 1999; Aramburu Guarda et al. 1999).

Materials and Methods

Study Site. Mosquitoes were collected at the village of Puerto Almendra and a densely forested site ≈0.3 km from the village, ≈20 km west-southwest of Iquitos (approximate population 300,000), Loreto Department, in the Amazon Basin region in northeastern Peru, ≈125 m above sea level and bordered by the Amazon, Itaya, and Nanay Rivers (3°49′ S, 73°22′ W).

The rural village of Puerto Almendra consists of single-dwelling homes located ≈0.3 km from the forest study site described by Jones et al. (2004). Dogs and chickens were the most common peridomestic animals observed in the village. Feral animals (i.e., squirrel monkeys [*Saimiri* spp.], woolly monkeys [*Lagothrix* spp.], bandicoots [*Nassua* spp.], macaws [*Ara* spp.], and parrots [*Amazona* spp.]) were kept as pets by some of the local residents. The local population included hunters and gatherers who have virtually hunted out most of the wild game and large rodents. Animals in forested areas surrounding Puerto Almendra include rodents (*Proechimys* spp., *Oryzomys* spp., and *Neacomys* spp.), marsupials (*Philander* spp., *Metachirus* spp., and *Marmosops* spp.), and sloths (*Choloepus hoffmanni* and *Bradypus* spp.). Adjacent to the forest and <1 km from the collection site is open farmland with free-ranging water buffalo, cattle, and horses. Numerous birds, including parrots, were observed in the forest.

Mosquito Collections. A total of nine adult mosquito collections were conducted from October 1996 through September 1997 under an approved human use protocol. Collections were conducted at ≈6-wk intervals and each consisted of four to six 24-h collections (two 12-h intervals) over a 10- to 16-d period. Volunteers, wearing a hooded screened jacket to prevent biting on the upper parts of the body, conducted hourly human-landing collections using mouth aspirators for 40 min starting on the hour, followed by a 20-min rest break, from 0600 to 1740 hours (daytime collection) and from 1800 to 0540 hours (nighttime collection). Collectors were positioned both inside and immediately outside of houses in the village of Puerto Almendra and both on the ground and 10 m up in the canopy at the forested site.

In addition, to compare the relative efficiency of dry ice–baited traps and human-landing collections, mosquitoes were collected with dry ice–baited ABC light traps (American Biophysics, Greenwich, RI), operated for 12-h periods from 0600 to 1800 hours and from 1800 to 0600 hours, that were paired with human landing collections. Captured mosquitoes were placed in humidified coolers after each hourly collection, and at the end of each 12-h collection period, they were transported to a central laboratory in Iquitos where they were identified according to Lane (1953), Pratt (1953), Guerdes and Souza (1964), Bram (1967), Pecor et al. (1992), and Sallum and Forattini (1996). Mosquito species collected are listed in Pecor et al. (2000), and preliminary mosquito bionomic findings are presented in Jones et al. (2004). After identification, the culicine mosquitoes were pooled (25–50 specimens) according to species, placed in sterile 1.5-ml cryovials, and maintained on dry ice or at −70°C until assayed for pathogens (Turell et al. 2005). The anophelines were pooled (1–10 specimens) by species for malaria circumsporozoite detection by ELISA.

Voucher specimens were deposited in the Walter Reed Biosystematics Unit, Smithsonian Institution, Washington, DC, where our field mosquito identifications were confirmed (Pecor et al. 2000).

Research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, 1996. The facility where this research was conducted is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

Results

Around one half of a total of 12,533 mosquitoes collected from Puerto Almendra from October 1996 through September 1997 were *Anopheles* spp. (Table 1). Approximately 10 times as many mosquitoes were captured in collections at the forest site (Jones et al. 2004) than in paired collections at Puerto Almendra. However, despite the overall lower numbers collected in the village, >300-fold more *An. darlingi* were captured in Puerto Almendra than in the closely located forest site. *Mansonia indubitans/titillans* were similarly collected at both sites, whereas *Aedes* spp., *Culex*

Table 1. Mosquitoes captured in human landing collections indoors and outdoors at single family dwellings in the village of Puerto Almendra and at a forested area ~300 m from the village

	Puerto Almendra		Forest	
	No./d[a]	Percentage[b]	No./d[a]	Percentage[b]
Aedes spp.	7.4	9.9	235.7	32.9
Ae. fulvus (Wiedemann)	1.3	1.7	140.1	29.6
Ae. serratus (Theobald)	6.1	8.2	92.7	13.0
Anopheles spp.	37.6	50.4	2.3	0.3
An. darlingi	37.1	49.7	0.1	<0.1
Coquillettidia venezuelensis (Theobald)	1.9	2.5	6.1	0.9
Culex spp.	12.7	17.0	121.3	17.0
Cx. (*Cux.*) spp.	2.4	3.2	14.5	2.0
Cx. (*Mel.*) spp.	10.2	13.7	106.8	14.9
Mansonia indubitans/titillans	8.1	10.9	6.1	0.9
Psorophora spp.	3.7	5.0	303.0	42.3
Ps. albigenu (Peryassu)	3.2	4.3	262.4	36.7
Ps. ferox (von Humboldt)	0.4	0.5	38.0	5.3
Other species[c]	3.2	4.3	41.1	5.7
Total	74.6	100	715.6	100

[a] Mean no. of mosquitoes collected per 24-h period (40 min/h, indoor and outdoors in Puerto Almendra and on the ground at the forest site) per site (168 trap-days at Puerto Almendra and 84 trap-days at the forest site).
[b] Percentage of the mosquitoes captured at that site. Note that the percentage for each genus includes all of the species listed below it.
[c] Consists of 39 additional species.

spp., and *Psorophora* spp. were collected in much greater numbers at the forested site (Table 1).

Anopheline Composition and Distribution. A total of 10 *Anopheles* spp. from three subgenera were collected in human landing collections (Table 2). *An. darlingi* was the most commonly captured mosquito species in the village and accounted for nearly 99% of the *Anopheles* captured in the village, whereas *Anopheles kompi* Edwards and a member of the *Anopheles mediopunctatus* (Theobald) complex (*Anopheles forattini* Wilkerson and Sallum) were the most commonly collected *Anopheles* spp. in the nearby forest (Table 2).

Comparison of Mosquitoes Captured Inside and Outside of Houses. Paired human collectors captured mosquitoes inside and outside houses at Puerto Almendra for 88 person-days each to determine which mosquito species entered dwellings. Each of the commonly collected mosquito species readily entered the relatively open wooden structure houses, with between 28 and 60% of the specimens captured indoors for the various species (Table 3). Despite differences in seasonal distribution and relative abundance, similar numbers of *An. darlingi* were captured indoors (51.0%) and outdoors (49.0%) (Table 1; Fig. 1).

Comparison of the Number of Mosquitoes Captured at Human Collectors and at Dry Ice–baited Light Traps. Paired human collectors and dry ice–baited light traps located inside and outside houses at Puerto Almendra for 168 trap nights were compared with determine the relationship between mosquitoes captured in dry ice–baited light traps with those that came to human collectors. Some species, such as *An. darlingi*, were collected almost exclusively by the human collectors, whereas others, such as the *Culex species*, were preferentially collected in the dry ice–baited light traps (Table 4). The various *Aedes*, *Psorophora*, and *Coquillettidia* species were readily collected by both methods.

Table 2. *Anopheles* spp. collected in human landing collections in the village of Puerto Almendra and at a forested site located ~300 m from the village from Oct. 1996 through Sept. 1997

Species	No. adults females collected (% by site)[a]		
	Puerto Almendra	Forest	Totals
An. (*Nys.*) *darlingi*	6,225 (98.7)	20 (5.3)	6,245 (93.4)
An. (*Ano.*) *forattini*	32 (0.5)	150 (39.7)	182 (2.7)
An. (*Ste.*) *kompi* Edwards	1 (<0.1)	163 (43.1)	164 (2.5)
An. (*Nys.*) *triannulatus* (Neiva and Pinto)	7 (0.1)	23 (6.1)	30 (0.4)
An. (*Nys.*) *oswaldoi* (Peryassu)	7 (0.1)	18 (4.8)	25 (0.4)
An. (*Nys.*) *nuneztovari* Gabaldon	13 (0.2)	9 (2.4)	22 (0.3)
An. (*Ano.*) *shannoni* Davis	7 (0.1)	9 (2.4)	16 (0.2)
An. (*Nys.*) *benarrochi* Gabaldon, Cova Garcia, and Lopez	7 (0.1)	2 (0.5)	9 (0.1)
An. (*Ano.*) *mattogrossensis* Lutz and Neiva	5 (0.1)	3 (0.8)	8 (0.1)
An. (*Ano.*) *peryassui* Dyar and Knab	6 (0.1)	0 (0)	6 (0.1)
Total	6,310	397	6,688

[a] Sum of 168 24-h collections (40 min/h, indoor and outdoors at Puerto Almendra and on the ground and in the canopy at the forest site.

Table 3. Comparison of mosquitoes captured inside and outside of houses in the village of Puerto Almendra

Species	Number collected[a]		% indoors
	Indoors	Outdoors	
Anopheles darlingi	3,305 (37.6)	3,174 (36.1)	51.0
Coquillettidia venezuelensis	126 (1.5)	202 (2.4)	38.5
Culex (*Culex*) spp.	109 (1.3)	286 (3.4)	27.7
Culex (*Mel.*) spp.	605 (7.2)	1,126 (13.4)	35.0
Mansonia indubitans/titillans[b]	932 (11.1)	622 (7.4)	60.0
Aedes serratus	462 (5.5)	857 (10.2)	35.0
Psorophora albigenu	252 (3.0)	294 (3.5)	46.2

[a] Total (mean) no. of mosquitoes captured per 24-h period by paired human collectors (one inside and one outside of a house) for 88 person-days at each location.
[b] These two species were not differentiated.

Table 4. Comparison of the no. of mosquitoes captured at human collectors and at dry ice–baited light traps in the village of Puerto Almendra

Species	No. mosquitoes collected[a]		% at humans
	Human collectors	CO_2-baited light traps	
Anopheles darlingi	55.2	1.9	97
Coquillettidia venezuelensis	3.6	1.7	68
Culex (*Culex*) spp.	5.1	16.6	24
Culex (*Mel.*) spp.	23.6	184.2	11
Mansonia indubitans/titillans[b]	9.7	2.0	83
Aedes serratus	8.6	3.5	71
Aedes fulvus	2.6	2.8	48
Psorophora albigenu	5.2	3.5	60

[a] Mean no. of mosquitoes captured per 24-h period by paired human collectors and dry ice–baited miniature light traps for 168 person-days. The numbers captured by the human collectors were multiplied by 1.5 to adjust for their collections only being 40 min/h.
[b] These two species were not differentiated.

Seasonal Activity. Although *An. darlingi* was collected throughout the year during this study, populations were generally higher from October to April and very low from May to September (Fig. 1). Peak activity was in April, when a mean of 171.2 (=256.8 after adjustment for only 40 min/h collection periods) *An. darlingi* was captured by each human collector. In contrast, a mean of only 2.0 (=3.0 after adjustment for 40 min/h collection periods) was captured during July through September.

Biting Activity. *Anopheles darlingi* biting activity was unimodal, with landing activity abruptly increasing at 1800 hours, peaking at 2000 hours, and slowly declining until 0600 hours. Host-seeking activity was similar for outdoor and indoor collections throughout the 24-h cycle (Fig. 2).

Discussion

Anopheles darlingi was the most prevalent mosquito captured by human collectors in the rural village studied but was rarely collected at a forested site located 300 m away. Although dry ice–baited light traps efficiently collected *Culex*, *Aedes*, and *Psorophora* spp., human landing collections were significantly more efficient at capturing *An. darlingi* and *Mansonia indubitans/titillans* [*M. indubitans* Dyar and Shannon and *M. titillans* (Walker) were not differentiated but were found in about equal numbers; Jones et al. 2004]. All of the commonly collected species readily entered houses because collectors located inside and outside of houses captured similar numbers of specimens for each species. This is partially because of the types of relatively open wooden structure dwellings. Because of the warm temperatures, the occupants frequently left the windows open for ventilation, or in some cases, doors were left open or were lacking, allowing mos-

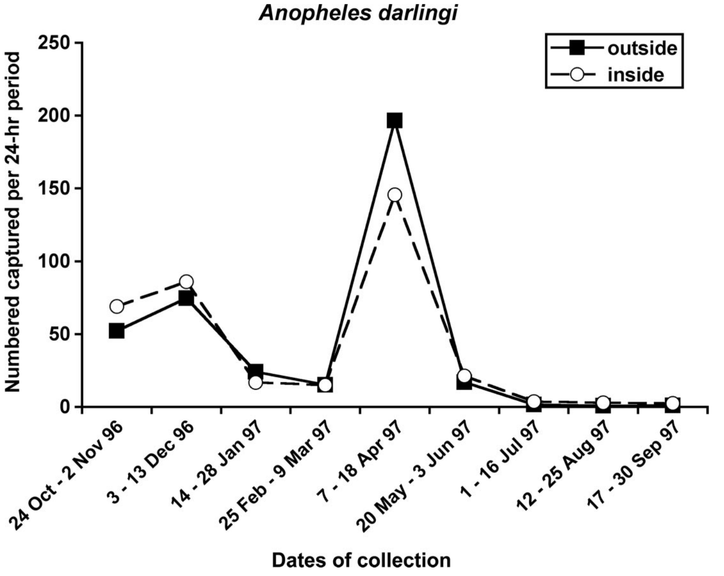

Fig. 1. Number of *An. darlingi* captured by paired collectors (one inside and one outside a house) for each collection period (8–12 24-h collections made inside and outside during each period) from October 1996 through September 1997.

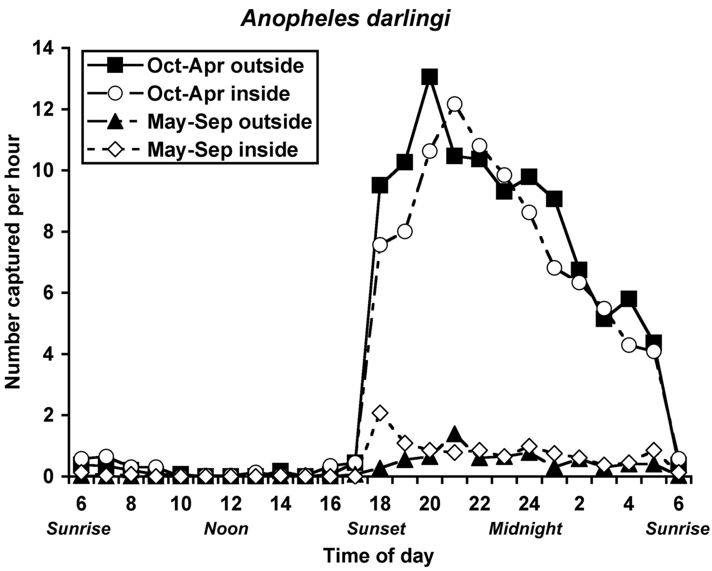

Fig. 2. Time of day of *An. darlingi* activity in the village of Puerto Almendra. Mosquitoes were captured by paired collectors (one inside and one outside) per house for 88 24-h periods each. Each collection lasted for 40 min and began on the hour. The numbers captured were multiplied by 1.5 to calculate the number per hour.

quitoes to easily enter the houses and bite the occupants.

Since 1991, the Amazon Basin region of the Loreto Department of Peru has experienced an unprecedented increase in the incidence of malaria, with the reported number of *Plasmodium falciparum* cases increasing from 140 in 1991 to 54,290 slide-confirmed cases in 1997 (Aramburu Guarda et al. 1999). The reasons for the increase has not been clearly established; however, in addition to ecological changes (e.g., deforestation), the recent spread of *An. darlingi* throughout the Department of Loreto has been cited as a leading factor (Aramburu Guarda et al. 1999, Schoeler et al. 2003). *An. darlingi* has been incriminated as the principal malaria vector throughout much of the Amazon Basin (Causey et al. 1946, Roberts et al. 1987, Klein and Lima 1990). Furthermore, it has been shown to be a very efficient vector of both *P. falciparum* and *Plasmodium vivax* malaria in Brazil (Klein et al. 1991a, b). According to Need et al. (1993), *An. darlingi* were not observed during a comprehensive longitudinal mosquito survey conducted in the Iquitos area from 1988 to 1991 and was first reported by Fernandez et al. (1996) from the Iquitos area of Peru in a 1994 larval/adult survey. By 1996, *An. darlingi* was the predominant anopheline mosquito collected in human-landing collections in most rural areas and villages surrounding Iquitos.

Studies have indicated an association between disturbed habitats and the relative abundance of *An. darlingi* (Walsh et al. 1993) and have hypothesized that recent increases in the prevalence of malaria is caused by ecological modifications of the environment, e.g., deforestation, in the Amazon Basin (Vittor et al. 2006). We captured significantly higher numbers of *An. darlingi* in Puerto Almendra than in the closely located forested area, despite the fact that the forested area was well within the 7-km flight range reported for this species (Charlwood and Alecrim 1989). In the forested area, of the 20 *An. darlingi* captured (Jones et al. 2004), only two were collected in the canopy. Thus, whereas this species could be found in the canopy, nearly all were captured at ground level. This confirms earlier studies by Roberts et al. (1987) and Charlwood and Alecrim (1989) that *An darlingi* are more frequently collected at houses in a village than at either the forest edge or the forest itself. Therefore, the increased trapping of *An. darlingi* in disturbed areas may be more related to where they seek a bloodmeal than to increased larval habitat, mats of floating debris, or floating vegetation in areas inundated by rivers and streams (Rozendaal 1992, Manguin et al. 1996).

Malaria has long been recognized as a problem in the Amazon Basin region of Peru, and at least 95,000 cases were confirmed Peru in 1944 before the initiation of a malaria control program and introduction of DDT that reduced the number of reported cases to 1,500 in 1965. Rozendaal et al. (1989) showed that DDT is effective against *An. darlingi.* The resurgence of malaria reported since 1991 is probably caused by an increased prevalence of *An. darlingi;* however, the rapid increase in the prevalence of this species may be caused, in part, by changes in mosquito control strategies, i.e., use of more costly and less effective rapidly biodegradable pesticides.

We collected similar numbers of *An. darlingi* both inside and outside of human habitations and captured significantly more specimens that attempted to land on human collectors than in dry ice–baited light traps. This confirms that, in this area, *An. darlingi* is both anthropophilic and endophilic, as has been reported (Roberts et al. 1987, Deane 1988, Klein and Lima

1990). Additionally, the high percentage of anophelines biting indoors is likely a reflection of the open wooden house construction, unscreened windows and doors, and close association with forest habitats, streams, and ponds.

The nocturnal biting activity of *An. darlingi* corresponds to human activity (Lourenco-de-Oliveira et al. 1989). Peak biting activity occurs in the early evening when people are active both outdoors and indoors. However, over its range, the peak biting activity of *An. darlingi* is variable, ranging from unimodal (i.e., early evening to 0200 hours), bimodal (i.e., early evening and early morning), and trimodal (i.e., early evening, late evening, and early morning) (Deane et al. 1948, Elliott 1972, Charlwood and Hayes 1978, Roberts et al. 1987, Rozendaal 1987, Klein and Lima 1990). Although most studies indicated a bimodal biting peak (early evening and morning), the biting activity near Iquitos was unimodal, with only an early evening biting peak. Deane et al. (1948) showed a single indoor biting peak that did not correspond with the outside activity. However, indoor and outdoor biting activity were similar in our study and may be caused by the type of house construction, because most houses were made of wood with large openings in the eaves and with unscreened windows and doors. In similar types of housing in Rondonia, Brazil, and in Padre Cocha, Loreto Department, Peru, there were no differences between indoor and outdoor biting activities reported (Klein and Lima 1990, A. Chan unpublished data). Variations in biting behavior may be caused by human population activity variation throughout the wide range of *An. darlingi* (Deane 1988).

The methods and locations of mosquito collection may significantly affect the catch size and species detected. For example, moving only ≈300 m from a relatively open area to a densely forested area in our study produced a dramatic change in both the species and numbers of mosquitoes collected, although collections were conducted in the same manner on the same nights. More than 10-fold more mosquitoes were captured in the forested area, and yet *An. darlingi*, the most prevalent mosquito in the village ≈300 m away, was virtually not seen, and it is possible that previous studies using dry ice–baited light traps in a forested area failed to detect this species. Similarly, time-of-year of collection can significantly affect trapping success. In our study, *An. darlingi* was infrequently collected, despite extensive efforts by human collectors, from May through September, with a mean of only 6.3 collected per 24 h by human collectors. In contrast, a mean of 69.5 was collected per 24 h from October through April, and 171.1 per 24 h were collected during the peak month of April 1997.

Although conducting human landing collections are more labor intensive than using dry ice–baited miniature light traps and have increased risks of exposing individuals to infectious diseases, these collections provide more accurate information on relative biting rates that can be better correlated with disease transmission risks. In this study, the use of only dry ice–baited light traps would have significantly underestimated the presence of *An. darlingi* in Puerto Almendra and significantly overestimated the risk of bites by *Culex* spp. mosquitoes. Although these traps often do not provide a good estimate of relative biting activity, they are useful in capturing large numbers of mosquitoes for identifying arboviruses. Additionally, after comparative trials between human and trap collections, correlations may be established to estimate human biting rates from trap collection data.

Mosquitoes have been implicated or suspected in the enzootic and epizootic transmission of numerous pathogens affecting human health in cities and rural villages of the Amazon Basin. Testing of the culicine mosquitoes captured during this and the companion study in the forested area (Jones et al. 2004) indicated that they contained numerous arboviruses. Turell et al. (2005) isolated viruses from 166 separate pools of mosquitoes, including eastern equine encephalitis virus (EEEV), Venezuelan equine encephalitis virus (VEEV), St. Louis encephalitis virus, and a variety of bunyaviruses. Further evaluation of live mosquitoes captured in conjunction with these studies indicated that *Culex* (*Melanoconion*) *gnomatos* Sallum, Hutchings, and Ferreira was the principal vector of VEEV (Turell et al. 2000, 2006) and that *Culex* (*Melanoconion*) *pedroi* Sirivanakarn and Belkin was the principal vector of EEEV (Turell et al. 2008). In addition, evaluation of the anophelines captured in this study showed low numbers positive for circumsporozoite antigen for both *P. falciparum* and *P. vivax* (T.A.K., unpublished data), and an overall circumsporozoite rate of 0.077% was detected in *An. darlingi* captured in the nearby village of Padre Cocha (≈10 km from Puerto Almendra) (A. Chan, unpublished data).

The emergence and expansion of enzootic and zoonotic diseases is particularly sensitive to ecological changes, population movements, and immigration of humans into sylvatic environments. Urbanization, agricultural development, and deforestation of the Amazon Basin have increasingly exposed human populations to previously known and unknown enzootic and zoonotic pathogens. Furthermore, ecological changes resulting from the invasion of humans into remote areas surrounding Iquitos, Peru, rustic living conditions, and limited health resources, in part, have resulted in increased numbers of arthropod-borne illnesses. A better understanding of potential vectors present, periods of activity, relative efficiency of various trapping methods, pathogens which with they are naturally infected, and which of these pathogens they can transmit (i.e., vector competence) is needed so that appropriate control strategies can be developed that target vector populations.

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