

Biting Rates and Developmental Substrates for Biting Midges (Diptera: Ceratopogonidae) in Iquitos, Peru

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J. Med. Entomol. 40(6): 807-812 (2003)

ABSTRACT Biting midges (Diptera: Ceratopogonidae) were collected at 16 periurban and rural sites around Iquitos, Peru, between 17 October 1996 and 26 May 1997. *Culicoides paraensis* (Goeldi), the principal vector of Oropouche virus, was the most commonly collected species (9,086 flies) with *Culicoides insinuatus* Wirth & Blanton second (7,229 flies). Although both species were collected at all sampling sites (linear distance surveyed \approx 25 km), *C. paraensis* dominated at northern collection sites (>90%), whereas *C. insinuatus* prevailed at southern collection sites (>60%). *C. paraensis* were collected from human sentinels at a constant rate throughout daylight hours, at similar rates during wet and dry months, and regardless of rainfall. Larval developmental substrates for *C. paraensis* included decaying *platano* (*Musa* \times *paradisiaca* L. [Musaceae]) stems, stumps, flowers, fruits, and debris beneath *platano* trees as well as from soil beneath a fruiting *mamay* (*Syzygium malaccense* Merr. & Perry [Myrtaceae]) tree and organic-rich mud along a lake shoreline. *C. insinuatus* adults likewise emerged from decaying *platano* and organic-rich mud along a lake shoreline, but also from debris accumulated in the axils of *aguaje* (*Mauritia flexuosa* L. [Palmae]) fronds and decaying citrus fruit. Despite high numbers of biting adults near putative substrates, adults of neither species emerged from other decomposing plant material, soil, phytotelmata, or artificial containers. Because both species of biting midges emerged in high numbers from all parts of *platano* (ubiquitous in Iquitos), it will be challenging to control them through sanitation.

KEY WORDS Oropouche virus, arbovirus, host-seeking, biting rates, developmental substrates

ORPOUCHE FEVER IS THE most important emerging human viral disease vectored by ceratopogonid biting midges in the Western Hemisphere. The causative agent, Oropouche (ORO) virus, is a member of the Simbu serogroup in the family Bunyaviridae. ORO virus causes a self-limited, dengue-like illness, characterized by fever, headache, myalgia, arthralgia, dizziness, anorexia, and prostration of 2- to 5-d duration (LeDuc et al. 1981). One to several recurrences of fever and chills occur in some patients after recovery. Effects upon fetal development have not been sufficiently studied, although other Simbu serogroup viruses cause abortions and teratogenic effects in animals (Linley et al. 1983).

ORO was first isolated from a charcoal maker in Trinidad in 1955 (Anderson et al. 1961); the first outbreak was documented in Belém, Para State (Brazil), in 1961 (Pinheiro et al. 1976). Subsequently, other outbreaks have also been reported from Panama

(1989) and Peru (1992), with >400,000 estimated cases worldwide between 1961 and 1995 (Tesh 1994). Additional outbreaks of ORO are likely in other regions of the Caribbean, Central America, and tropical South America as human populations grow, especially as they alter natural landscapes (Tesh 1994).

Culicoides paraensis (Goeldi) (Diptera: Ceratopogonidae) is the principal vector of ORO in periurban cycles (Dixon et al. 1981, LeDuc et al. 1981, Roberts et al. 1981, Hoch et al. 1986, Wirth and Felipe-Bauer 1989) and a possible vector of filarial worms (Shelley and Coscarón 2001) to humans in part of its range. The species is distributed from northern Argentina to north central United States (Wirth and Blanton 1973, Wirth and Felipe-Bauer 1989). Larvae have been collected from decaying plant tissue (*platano* [*Musa* \times *paradisiaca* L. (Musaceae)] stems and *cacao* [*Theobroma cacao* (L.) (Sterculiaceae)] hulls) and phytotelmata (Linley et al. 1983, Hoch et al. 1986, Wirth and Felipe-Bauer 1989, Hoch et al. 1990). Therefore, adults may be numerous in periurban settings with backyard gardens. Hoch et al. (1990) reported the daily biting activity of *C. paraensis* in Belém, Brazil; biting was highest during crepuscular periods in houses and sun-exposed sites, but peaked at midday in shaded peridomestic sites. Linley et al.

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Table 1. Collection sites of biting Ceratopogonidae in Iquitos, Peru, between 17 October 1996 and 26 May 1997

| Site | Location | Description |
|-----------------|----------------------------|--|
| Santa Maria | S03° 42.001' W073° 14.397' | Small village, farms, backyard gardens, seasonal floodplain of Amazon River |
| Punchana | S03° 43.102' W073° 14.361' | River port, periurban, gardens, stilted homes on Amazon River floodplain |
| Padre Isla | S03° 43.523' W073° 13.769' | Island in Amazon River channel, rural with dense gardening and secondary forest |
| Moronillo | S03° 43.784' W073° 15.653' | Periurban, heavily residential surrounding seasonally flooded lagoon |
| Pradera | S03° 44.035' W073° 16.198' | Edge of periurban settlement along lagoon of Nanay River |
| Moronococha | S03° 44.625' W073° 15.938' | Densely populated periurban neighborhood above lagoon of Nanay River |
| Avenida Brasil | S03° 44.830' W073° 15.407' | Periurban, heavily populated with drainage streams |
| Aserradero | S03° 45.422' W073° 16.382' | Private orchard with cement works adjacent to sawmill drainage stream |
| Belen | S03° 45.925' W073° 15.139' | Densely populated periurban stilted neighborhood seasonally flooded by Itaya River |
| Santa Clara | S03° 46.937' W073° 20.465' | Isolated village along Nanay River, backyard gardens, surrounded by secondary forest |
| Vihau | S03° 48.131' W073° 18.513' | Small rural neighborhood, farms, less standing water |
| Finca de Sabino | S03° 49.168' W073° 19.316' | Rural farm and orchard, limited standing water, surrounded by secondary forest |
| Finca de Juan | S03° 49.652' W073° 20.105' | Rural farm and palm plantation, surrounded by secondary forest |
| Quistococha | S03° 49.760' W073° 19.310' | Oxbow lake and tourist zoological center with sandy beach, secondary forest |
| Correntillo | S03° 49.935' W073° 21.742' | Dark water lagoon, surrounded with palm plantations, pasture, and secondary forest |
| Morolillo | S03° 54.626' W073° 20.336' | Isolated village, relatively recent, small farms with primary and secondary forest |

Sites are ordered from north to south.

(1983) compiled data indicating peak biting activities during late afternoon.

Iquitos, Peru, a port city on the Amazon River between the confluences of the Nanay and Itaya Rivers with >300,000 residents, was the site of a recent outbreak of ORO fever. Serological surveys in Iquitos have indicated high ORO antibody rates and annual transmission among residents of Iquitos (Tesh 1994, Watts et al. 1997, Baisley et al. 1998). Iquitos serves as a tourist, trade, and military center for the northern part of Amazonian Peru. The region has also hosted numerous cooperative studies among Peruvian military and public health agencies and United States researchers. Therefore, Iquitos not only serves as a locus for the spread of ORO, but it also presents a unique opportunity to study an emerging arbovirus near its edge of expansion.

The prevalence of ORO virus infection apparently increases with age-related exposure to bites from *C. paraensis*. A serological survey of ORO antibodies in Santa Clara (a small village near Iquitos) indicated a low incidence of infection for children (6–7%), but >50% incidence for adults (25+ years, overall incidence 34%, Baisley et al. 1998). Conversion of tropical rain forest to agricultural use may favor the development of *C. paraensis* by providing more immature developmental sites and human blood sources. As Iquitos expands and human colonizers clear the rain forest to build homes and plant gardens, they encounter forest insects and novel pathogens. We studied biting patterns and developmental substrates in periurban and rural sites around Iquitos to evaluate the vector potential of *C. paraensis* for ORO virus transmission.

Materials and Methods

Adult Collections. All observations were conducted in Iquitos, Department of Loreto, Peru, between 17 October 1996 and 26 May 1997. Collections were made at 16 rural and periurban sites that are described in Table 1. Ceratopogonidae were captured as they attempted to bite the exposed ankles or forearms of collectors. Attentive collectors captured flies as they

landed, and all collecting was halted before sunset to avoid malarial vectors. All biting flies were aspirated by mouth, transferred to screened specimen cups, and provided with cotton moistened with sugar water. Biting midges were killed by freezing, counted, and identified (by D.R.M.) using a dissecting stereomicroscope according to published keys (Wirth and Blanton 1973, Wirth et al. 1988, Wirth and Felipe-Bauer 1989) and a reference collection of local species (provided by G.R.S.). Rare individuals were identified using wing patterns and genitalia dissection (by G.R.S.).

During August 2000, a Garmin GPS III Plus (Garmin Corporation, Olathe, KS) was used to estimate the latitude and longitude for each collection site. The ratio ($C. paraensis / [C. paraensis + C. insinuatius]$) captured was calculated for each collection site for all dates. We investigated significant changes in the species ratio of biting flies for each site along latitudinal and longitudinal transects using Pearson's product-moment correlation coefficients (Sokal and Rohlf 1981). All statistical tests were performed with Statistica (1995).

Monthly Collections. Between January and May 1997, monthly ceratopogonid collections were conducted at 12 sites, a representative subset of the 16 sites used. Two collectors at each site collected all biting midges between 3:45 and 5:45 p.m. Three nearby sites were visited each day for each of 4 consecutive days each month. Analysis of variance (ANOVA) was used to test significant differences ($\alpha = 0.05$) among months of collection and among sample sites for total numbers of biting midges (Sokal and Rohlf 1981).

Hourly Collections. Five collectors captured all biting midges seeking blood meals for 40 min each hour between 7:00 a.m. and 5:40 p.m. for 1 d at each of six sites. The collectors rotated among five collection stations at each site; the stations were representative of outdoor locations used by local residents (e.g., doorways, backyard gardens) and were separated by at least 50 m. Between collections, the collectors moved to a rest station distant from any of the collection

Table 2. Numbers of *C. paraensis* and *C. insinuatus* collected biting humans at 16 sites in Iquitos, Peru

| Site | Collections | <i>C. paraensis</i> | <i>C. insinuatus</i> | % <i>C. paraensis</i> |
|-----------------|-------------|---------------------|----------------------|-----------------------|
| Santa Maria | 5 | 1,831 | 7 | 99.62 |
| Punchana | 12 | 776 | 3 | 99.61 |
| Padre Isla | 9 | 3,619 | 153 | 95.94 |
| Moronillo | 5 | 137 | 5 | 96.48 |
| Pradera | 6 | 170 | 17 | 90.91 |
| Moronococha | 5 | 14 | 4 | 77.78 |
| Avenida Brasil | 5 | 102 | 3 | 97.14 |
| Aserradero | 8 | 673 | 3 | 99.56 |
| Belen | 5 | 53 | 13 | 80.30 |
| Santa Clara | 11 | 440 | 104 | 80.88 |
| Vihau | 1 | 44 | 625 | 6.58 |
| Finca de Sabino | 12 | 1,015 | 1,911 | 34.69 |
| Finca de Juan | 10 | 63 | 226 | 21.80 |
| Quistococha | 7 | 20 | 3,818 | 0.52 |
| Correntillo | 3 | 4 | 135 | 2.88 |
| Morollillo | 3 | 125 | 202 | 38.23 |
| Total | 107 | 9,086 | 7,229 | 55.69 |

Collections made October 1996 to May 1997.
 Sites are ordered from north to south.

stations. ANOVA was used to test significant differences in total biting midge captures among sample sites and hour of collection (Sokal and Rohlf 1981).

Larval Developmental Substrates. Developmental substrates were identified by placing field-collected materials in darkened boxes and allowing emergent adults to fly into naturally lighted collection containers. Materials tested included fresh and decaying vegetation, soil, and standing water collected from 11 of the sites used for adult collection. Putative developmental substrates were carried to the laboratory in sealed plastic bags, aspirated with a backpack-powered aspirator to remove any extraneous insects, placed into plastic trays, and enclosed in tiers of boxes. The boxes were constructed of plywood, sealed along all seams with silicon, and painted with flat black enamel paint on the inside. A perforated lid of a specimen cup was attached to the top of each box. Emergent insects were collected daily from the lighted specimen cup. Collections were halted when 5 d passed without additional insect emergence.

Results

Adult Collections. A total of 16,503 host-seeking Ceratopogonidae was captured on 57 dates. Of these, 9,086 (55.06%) were *C. paraensis* and 7,229 (43.80%) were *C. insinuatus* Ortiz & Leon. Very few other biting midges were captured. Of these, 63 (0.38%) were *Culicoides daviesi* Wirth & Blanton, 33 (0.20%) were *Culicoides fluviatilis* (Lutz), and 92 could not be identified. Because these other species constituted <1% of the total ceratopogonid collected and identified, they were not included in analyses.

C. paraensis and *C. insinuatus* were captured at every site, but the relative proportions of these two species changed significantly along north-south and east-west transects (Table 2). The proportion of *C. paraensis* decreased significantly from north to south ($r = -0.80$) and east to west ($r = -0.81$). The linear distance between the northernmost and southernmost points sampled is ≈ 25 km (<http://jan.ucc.nau.edu/~cvm/latlongdist.html>).

Monthly Collections. Mean numbers of biting midges captured did not differ among the 5 mo of collection for *C. paraensis* ($F_{4,55} = 0.86, P = 0.50$ by ANOVA, Table 3; these data included in totals from Table 2), *C. insinuatus* ($F_{4,55} = 0.70, P = 0.60$), or both species ($F_{4,55} = 1.14, P = 0.35$). However, sample sites produced unequal numbers of *C. paraensis* ($F_{11,48} = 6.02, P < 0.0001$, Table 3), *C. insinuatus* ($F_{11,48} = 2.86, P < 0.01$), and combined biting midges ($F_{11,48} = 3.02, P < 0.01$). Santa Maria and Padre Isla produced significantly more *C. paraensis* (by post hoc comparisons of means by Scheffé's test), and Quistococha and finca de Sabino produced significantly more *C. insinuatus* than the remaining sites across all dates.

Hourly Collections. Mean biting rates for *C. paraensis* ($F_{10,53} = 0.15, P = 0.99$) and *C. insinuatus* ($F_{10,53} = 0.68, P = 0.74$) did not vary during the hours of collection ($F_{10,53} = 0.81, P = 0.624$ for both species, Table 4). Mean numbers ($F_{5,58} = 17.48, p \ll 0.001$) and relative proportions of biting midges differed significantly among collection sites. Padre Isla, finca de Sabino, and Quistococha produced significantly more

Table 3. Numbers of *C. paraensis* and *C. insinuatus* collected biting humans at 12 sites in Iquitos, Peru, between 3:45 and 5:45 p.m.

| Site | January | February | March | April | May | Totals |
|-----------------------|----------|-------------|---------|---------|---------|-------------|
| Santa Maria | 350/1 | 513/0 | 392/4 | 60/2 | 129/0 | 1,444/7 |
| Padre Isla | 424/0 | 210/0 | 60/43 | 4/10 | 91/131 | 789/184 |
| Punchana | 108/0 | 71/0 | 43/1 | 132/1 | 29/1 | 383/3 |
| Moronillo | 5/0 | 17/0 | 45/0 | 6/2 | 22/1 | 95/3 |
| Pradera | 16/0 | 37/6 | 3/0 | 9/3 | 30/2 | 95/11 |
| Avenida Brasil | 14/0 | 28/1 | 14/0 | 17/1 | 1/0 | 74/2 |
| Moronococha | 1/1 | 0/0 | 3/1 | 5/2 | 4/0 | 13/4 |
| Belen | 0/0 | 1/0 | 18/0 | 12/0 | 6/9 | 37/9 |
| Aserradero | 25/0 | 79/0 | 48/1 | 31/1 | 49/0 | 232/1 |
| Santa Clara | 90/2 | 72/4 | 50/5 | 60/2 | 5/8 | 277/21 |
| Sabino | 31/15 | 96/59 | 172/154 | 70/35 | 53/228 | 422/526 |
| Quistococha | 1/32 | 0/1,172 | 1/326 | 1/95 | 3/180 | 6/1,805 |
| Total | 1,065/51 | 1,124/1,242 | 849/535 | 497/153 | 422/551 | 3,867/2,576 |
| % <i>C. paraensis</i> | 95.43 | 47.50 | 61.34 | 76.46 | 43.37 | 60.0 |

Data represent *C. paraensis*/*C. insinuatus*.

Table 4. Numbers of *C. paraensis* and *C. insinuatus* collected by five collectors during hourly collections from six sites in Iquitos

| Hour | Padre Isla 12 Feb 97 | Sabino 17 Feb 97 | Quistococha 20 Feb 97 | Aserradero 13 Mar 97 | Juan 18 Mar 97 | Vihau 21 May 97 |
|--------|-------------------------|---------------------|--------------------------|-------------------------|-------------------|--------------------|
| 7 a.m. | NC*** | NC*** | 1/19** | 14/0 | 15/12 | 4/92 |
| 8 | 244/2*** | 47/32 | 3/343 | 31/0 | 4/4 | 2/40 |
| 9 | 190/3 | 66/121** | 1/243** | 52/0 | 7/0 | 5/41 |
| 10 | 239/0* | 64/227* | 1/167* | 28/0 | 3/0 | 3/5* |
| 11 | 233/0 | 36/357 | 1/419 | 35/0 | 9/0 | 6/66 |
| noon | 185/0* | 51/187* | 1/183 | 21/0 | 3/1 | 3/39 |
| 1 p.m. | 276/0 | 58/83 | 0/7*** | 28/1* | 3/0 | 3/7 |
| 2 | 179/0* | 23/24 | 1/90* | 14/0 | 0/1 | 6/74 |
| 3 | 272/0 | 42/114 | 0/213 | 20/0 | 2/16 | 3/129 |
| 4 | 277/0* | 35/165 | 1/236 | 16/0 | 1/34 | 2/69 |
| 5 | 155/0* | 27/76 | 4/59 | 18/1 | 2/11* | 7/63 |
| Totals | 2,250/5 | 449/1,386 | 14/1,979 | 277/2 | 49/79 | 44/625 |

Blood-seeking flies collected 40 min/h. Data represent *C. paraensis*/*C. insinuatus*.

Symbols: NC, no collection. *, Light rain during collection period. **, Moderate rain during collection period. ***, Heavy rain during collection period.

biting midges than the other three sites (by post hoc comparisons, Table 4).

Developmental Substrates. Both *C. paraensis* and *C. insinuatus* emerged from field-collected decomposing

Table 5. Putative developmental substrates and yield of *C. paraensis* and *C. insinuatus*

| Substrate | Source | <i>C. paraensis</i> | <i>C. insinuatus</i> |
|----------------------------|-------------------------|---------------------|----------------------|
| <i>platano</i> | | | |
| rotted stem* | Aser, Isla*, Sab, Juan* | 5/8 | 4/8 |
| fresh stem | Mor | 0/2 | 0/2 |
| leafy shoot* | Mor* | 1#/1 | 0/1 |
| rotted stump* | Isla* | 1/1 | 1/1 |
| rotted flowers/ fruits* | Aser, Isla* | 4/5 | 2/5 |
| fresh flowers | Pun | 0/2 | 0/2 |
| debris beneath* | Sab* | 1#/2 | 0/2 |
| <i>aguaje</i> stem | Sab | 0/2 | 0/2 |
| Soil | | | |
| <i>aguaje</i> axil* | Sab, Juan*, Sab | 0/3 | 1#/3 |
| under <i>aguaje</i> | Quist, Sta | 0/3 | 0/3 |
| forest floor | Quist | 0/1 | 0/1 |
| under <i>mamaj</i> * | Prad* | 1/1 | 1#/1 |
| from garden | Sab | 0/1 | 0/1 |
| sand at lakeshore | Quist | 0/1 | 0/1 |
| lake mud*, grass | Quist, Sta* | 1#/6 | 1#/6 |
| lake muck, grass | Sta | 0/1 | 0/1 |
| flooded river bank | Prad | 0/2 | 0/2 |
| sawdust | Aser | 0/1 | 0/1 |
| Fruits | | | |
| rotten <i>guajahu</i> | Aser | 0/1 | 0/1 |
| rotted citrus* | Sab* | 0/2 | 1#/2 |
| <i>guaba</i> pods | Sta | 0/1 | 0/1 |
| cacao pods | Juan | 0/1 | 0/1 |
| <i>macambo</i> pods | Juan, Corr | 0/3 | 0/3 |
| Container water | | | |
| trecholes | Porv, Isla | 0/4 | 0/4 |
| artificial | Isla, Sab, Pun, Sta | 0/7 | 0/7 |

Data represent adult ceratopogonids produced/samples tested.

Symbols: Aser = Aserradero, Isla = Padre Isla, Sab = finca de Sabino, Juan = finca de Juan, Mor = Morolillo, Pun = Punchana, Sta = Santa Clara, Quis = Quistococha, Prad = Pradera, Por = Porvenir, Corr = Correntillo. **Bold type** and an asterisk (*) indicate positive substrates and sources. Pound sign (#) indicates only one individual emerged.

Scientific names: *aguaje* = *Mauritia flexuosa* L. (Palmae); cacao = *Theobroma cacao* (L.) (Sterculiaceae); citrus = *Citrus* sp. (Rutaceae); *guaba* = *Inga edulis* Mart. (Leguminosae); *guayaba* = *Psidium guajaba* L. (Myrtaceae); *macambo* = *Theobroma bicolor* Humb. & Bonpl. (Sterculiaceae); *mamaj* = *Syzygium malaccense* Merr. & Perry (Myrtaceae); *platano* = *Musa × paradisiaca* L. (Musaceae).

platano (*Musa × paradisiaca* L. [Musaceae]) collected from three of the six collection sites tested (Table 5). High numbers of adult *C. paraensis* and *C. insinuatus* (mean \pm SD = 4.84 \pm 5.24 biting midges/g dry weight substrate, $N = 5$) emerged from decomposing *platano* stems, flowers, and fruits over relatively long periods (29.0 \pm 16.6 d, $N = 11$, Table 6). The relative ratios between *C. paraensis* and *C. insinuatus* adults that emerged from decomposing *platano* stems, flowers, and fruits (Isla = 73.97%, Juan = 36.42%, Table 6) were similar to ratios of adults collected at human sentinels at Iquitos Island and finca de Juan (Isla = 95.94%, Juan = 21.80%, Table 2). Fresh, green *platano* material did not produce any biting midges.

A few biting midges emerged from mud or soil that was high in decaying organic material, but none emerged from substrate with low organic content (Tables 5 and 6). One adult of each species emerged from lakeside mud in Santa Clara. *C. paraensis* (6) and *C. insinuatus* (1) adults also emerged from soil under a *mamaj* (*Syzygium malaccense* Merr. & Perry [Myrtaceae]) tree. One *C. paraensis* adult emerged from straw and other debris mixed with the soil beneath a *platano* tree. Likewise, single adult *C. insinuatus* emerged from debris collected from an *aguaje* (*Mauritia flexuosa* L. [Palmae]) leaf axil and from decaying citrus (*Citrus* sp. [Rutaceae]) fruit on the ground (in a garden with *platano* and other fruit trees). However, no adults of either species emerged from other decomposing fruits, vegetation, sawdust, or water tested from 11 sites (Table 5). *C. daviesi* adults emerged from *aguaje* debris and soil beneath *aguaje* trees; likewise, host-seeking adults of this species were consistently collected in low numbers by sentinels under palm trees.

Discussion

Periurban and rural residents of Iquitos experience high biting rates by Ceratopogonidae in their homes, backyard gardens, and farms throughout daylight hours. *C. paraensis*, the principal vector of ORO, was captured at every site surveyed, but was most common in north-eastern sites near the Amazon River and its tributaries. Likewise, biting rates for *C. insinuatus* were particularly high at half of the sites surveyed, especially sites farthest

Table 6. Yields and duration of emergence by *C. paraensis* and *C. insinuatus* from positive developmental substrates from Iquitos, Peru

| Date collected | Source | Substrate | <i>C. paraensis</i> | <i>C. insinuatus</i> | Dry weight substrate | Biting midges/g dry substrate | Duration of emergence |
|----------------|--------|--------------------------|---------------------|----------------------|----------------------|-------------------------------|-----------------------|
| 3 Jan 1997 | Isla | platano stem | 449 | 191 | 45.6 g | 14.04 | 56 d |
| 3 Jan | Isla | platano stem | 78 | 48 | 44.9 g | 2.81 | 43 d |
| 3 Jan | Isla | platano stem | 39 | 0 | 15.4 g | 2.53 | 25 d |
| 27 Jan | Isla | platano flowers & fruits | 178 | 39 | 56.9 g | 3.81 | 36 d |
| 27 Jan | Isla | platano flowers & fruits | 6 | 1 | ND | | 8 d |
| 27 Jan | Isla | platano flowers & fruits | 10 | 0 | ND | | 19 d |
| 27 Jan | Isla | platano flowers & fruits | 33 | 0 | ND | | 28 d |
| 20 Mar | Juan | platano stem | 3 | 38 | 40.0 g | 1.03 | 36 d |
| 20 Mar | Juan | platano stem | 7 | 4 | ND | | 20 d |
| 20 Mar | Juan | platano stem | 53 | 68 | ND | | 47 d |
| 20 Mar | Juan | aguaje axil soil | 0 | 1 | 119.0 g | 0.01 | 1 d |
| 9 Apr | Mor | platano leafy shoot | 1 | 0 | ND | | 1 d |
| 11 Apr | Sab | debris beneath platano | 1 | 0 | ND | | 1 d |
| 11 Apr | Sab | citrus fruit | 0 | 1 | ND | | 1 d |
| 8 May | Sta | lake organic mud | 1 | 0 | ND | | 1 d |
| 8 May | Sta | lake organic mud | 0 | 1 | ND | | 1 d |
| 16 May | Prad | soil beneath mamay | 6 | 1 | ND | | 6 d |

Symbols: Isla = Padre Isla, Juan = finca de Juan, Mor = Morolillo, Sab = finca de Sabino, Sta = Santa Clara, Prad = Pradera, ND = not determined.

Scientific names: aguaje = *Mauritia flexuosa* L. (Palmae); citrus = *Citrus* sp. (Rutaceae); mamay = *Syzygium malaccense* Merr. & Perry (Myrtaceae); platano = *Musa × paradisiaca* L. (Musaceae).

from the rivers (Table 2). Although the vector status of *C. insinuatus* for ORO is unknown, this species is a vector of filariae in parts of its range (e.g., Colombia, Tidwell and Tidwell 1982), but apparently not others (e.g., Brazil, Shelley et al. 1980).

Local residents refer to human biting ceratopogonids as *manta blanca* (i.e., white cloak) based upon adult swarms that are dense enough to discolor the skin. During collection periods, biting activity was often high. For example, between 3:45 and 5:45 p.m. on 25 February and 2 April 1997, 401 and 389 Ceratopogonidae, respectively (>99% *C. paraensis*), were collected by individual sentinels in Santa Maria. Although anecdotal reports by residents of Iquitos suggested maximal biting midge activities during early morning and late afternoon hours, these observations were not supported by the data. Observations by local residents may better reflect awareness of biting midge activities during periods of rest. Hoch et al. (1990) reported movement by host-seeking *C. paraensis* in Belém, Brazil, during periods of rain from open areas to doorways of homes. These results are consistent with high biting rates during light to very heavy rains during our study.

Iquitos residents also reported that biting activity by ceratopogonids increased dramatically during the annual flood stages of local rivers. This trend was not observed in the data. Iquitos was relatively dry during January 1997. Intense seasonal rains occurred during February and March; between April and May 1997, the Amazon, Nanay, and Itaya Rivers were flooded, including backyard gardens. Most homes within the flood plains are built on stilts to accommodate annual flooding. During the same period, collections made at flooded sites (Padre Isla, Santa Maria, Punchana, and Belen) showed no increases in biting midge numbers (Table 3).

Between an initial visit to Santa Maria (15 August 1996) and the final collection (2 May 1997), floodwa-

ters of the Amazon River eroded large portions of the Santa Maria, including collection sites. Nonetheless, collections made near the receding riverbank consistently yielded an overwhelming majority of *C. paraensis* adults. By August 2000, the entire community had been destroyed, and the GPS reading was taken in the channel of the Nanay River.

Both Santa Maria and Padre Isla yielded high numbers of biting midges, which were overwhelmingly *C. paraensis*. Quistococha, which produced comparable numbers of biting midges, yielded >99% *C. insinuatus*. Collections at Quistococha were done along an artificial sandy beach next to an oxbow lagoon or in the surrounding secondary forest within a tourist zoological preserve. Although there were few cultivated *platano* within the preserve, there were numerous putative developmental sites (e.g., phytotelmata, organic-rich soil, decomposing fruits and vegetation) that, apparently, were more suited for the development of *C. insinuatus*. Likewise, other collection sites south and west of Iquitos yielded significantly more *C. insinuatus* adults than *C. paraensis*, although both species developed and emerged from *platano* during our investigation.

It is unclear whether the changes in species compositions along north-south and east-west transects were caused by a fixed natural environmental gradient (e.g., the southern sites are further from the rivers and may have been better drained than northern sites) or whether the differences in species compositions were caused by an advance by *C. paraensis* resulting from changes in human settlement and landscape usage. If human activities have determined species composition in Iquitos, the incidence of *C. paraensis* and ORO will most likely increase as rain forest is converted to settlements.

C. paraensis did not emerge from the majority of potential developmental substrates tested, although Ceratopogonidae have been reported to develop in

these substrates. However, *C. paraensis* emerged in high numbers from a ubiquitous substrate: decaying *platano* tissues. Adults did not emerge from freshly cut or well-decomposed *platano* stems, but they did complete development in decaying flowers, fruits, stems, and stumps. Adults of this species also emerged from soil collected beneath a fruiting *mamay* tree, but not from decaying *mamay* fruits. Therefore, *C. paraensis* have plentiful developmental sites in backyard gardens and larger plantations, and human blood sources are readily available. Likewise, despite hourly collections, the numbers of host-seeking biting midges were not depleted by sampling. As a result, future efforts to reduce the incidence of ORO infection through vector control of *C. paraensis* will require prolonged and universal participation of local residents.

Acknowledgments

This research was supported in part by National Institutes of Health Grant (AI) 39800. We appreciate the assistance of J. Mozambique, K. Block, H. Carabao, and the collectors and residents of Iquitos. The manuscript was prepared at the University of Northern Iowa.

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Received for publication 20 March 2003; accepted 15 July 2003.