



Diurnal biting periodicity of parous *Simulium* (Diptera: Simuliidae) vectors in the onchocerciasis Amazonian focus

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Abstract

We describe the hourly patterns of parous biting activity of the three main simuliid vectors of human onchocerciasis in the Amazonian focus straddling between Venezuela and Brazil, namely, *Simulium guianense* s.l. Wise; *S. incrustatum* Lutz, and *S. oyapockense* s.l. Floch and Abonnenc. Time series of the hourly numbers of host-seeking parous flies caught in five Yanomami villages during dry, rainy, and their transition periods from 1995 to 2001 were investigated using harmonic analysis (assuming an underlying circadian rhythm) and periodic correlation (based on Spearman’s r). Parous *S. guianense* s.l. showed a bimodal activity pattern, with a minor peak in mid-morning and a major peak at 16:00 h. *S. incrustatum* exhibited mainly unimodal activity during either early morning or midday according to locality. *S. oyapockense* s.l. bit humans throughout the day mainly between 10:00 and 16:00 h but also showed bimodal periodicity in some localities. Superimposed on the endogenous, species-specific daily cycles, parous activity showed variation according to locality, season, air temperature and relative humidity, with biting being promoted by warmer and drier hours during wet seasons/periods and reduced during hotter times in dry seasons or transitions. The results are discussed in terms of their implications for blackfly biology and ecology as well as onchocerciasis epidemiology and control. © 2005 Elsevier B.V. All rights reserved.

Keywords: *Simulium guianense* s.l.; *S. incrustatum*; *S. oyapockense* s.l.; Time-series; Harmonic analysis; Host-seeking activity; Onchocerciasis; Southern Venezuela

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1. Introduction

Human onchocerciasis transmission and severity is the result of continuous exposure of individual hosts to the bites of *Simulium* Latreille vectors of *Onchocerca volvulus* Leuckart. Consequently, quantification of the degree of host exposure, measured by vector density and the biting rate of flies on humans is important to parameterize models for transmission dynamics and control (Renz and Wenk, 1987; Dye, 1994; Basáñez et al., 2002). The most commonly used indicator of the degree of human exposure in onchocerciasis studies is the annual biting rate (Duke, 1968). This parameter, which describes the yearly number of flies attempting to obtain a blood-meal on a human, represents more truly a ‘landing’ than a ‘biting’ rate thus overestimating exposure. Being a summary measure, the annual biting rate gives no real indication of the temporal and spatial variation in both blackfly abundance and human exposure (Renz, 1987). Investigation of the monthly, daily, and hourly variation in blackfly biting activity, and in particular of the parous component of the fly population, would permit identification of months of the year and hours of the day during which transmission of onchocerciasis is potentially highest (Renz, 1987). Ideally, these studies should be accompanied by estimates of the amount of time that populations at risk spend on activities that expose them to vector bites during these months and hours, in order to obtain more accurate estimates of contact rates for transmission models (Bockarie and Davies, 1990; Renz et al., 1987).

We have already shown that three anthropophilic simuliid species are involved in onchocerciasis transmission in the Amazonian focus of southern Venezuela, namely *Simulium guianense* Wise s.l., *S. incrustatum* Lutz, and *S. oyapockense* Floch and Abonnenc s.l. Such studies have also revealed that spatial (altitude, locality) and temporal (seasonal) variation in the biting activity of these species, together with their contrasting vectorial efficiencies contribute differentially to exposure and transmission in this endemic focus (Basáñez et al., 1988; Vivas-Martínez et al., 1998; Grillet et al., 2001). *S. guianense* s.l. prevails during the transition between dry and wet seasons in the highland hyperendemic areas. In these areas, *S. guianense* s.l. acts as the main vector of *O. volvulus* despite its relatively low parous biting rate (median = 40 parous bites/person-day; range = 4–350), due to its high

vector competence (Takaoka et al., 1984; Basáñez et al., 1995; Grillet et al., 2000, 2001). *S. incrustatum* plays a secondary vectorial role in hyperendemic settings, predominating mainly during the rains, rainy-dry and early dry seasons when its parous (and probably survival) rates are highest (median = 180 parous bites/person-day; range = 60–390) (Grillet et al., 2001). *S. oyapockense* s.l. is the prevalent species at lowland hypo- and mesoendemic forested areas, where it bites in very large numbers (median = 1920 parous bites/person-day; range = 1500–3300) during the dry and rainy seasons, compensating for its low vector competence (Shelley et al., 1987; Basáñez et al., 1988, 1995; Vivas-Martínez et al., 1998; Grillet et al., 2000, 2001).

Our results have also suggested that seasonal fluctuations of parous populations are correlated mainly with river level, with the dry season and the transition periods between seasons potentially contributing most to onchocerciasis transmission (Grillet et al., 2001). Less research has been conducted, however, on the patterns of hourly variation in biting activity for these simuliid species. Knowledge of these patterns would be important not only to help quantifying exposure but also to aid the design of sampling protocols that would maximize the efficiency of rapid entomological assessment (REntA) methods for the monitoring and evaluation of ivermectin-based control programmes (Basáñez et al., 1998; Vieira et al., 2005).

Blackflies exhibit a 24-h pattern of rest and activity, presumably driven by natural circadian rhythms timed or adapted to the predictable daily cycle of light and darkness, but readily modifiable by (exogenous) weather and environmental conditions, such as wind speed, rainfall pattern, temperature and humidity (Wenk, 1981; Crosskey, 1990). Adult females bite from dawn to dusk, but most activity occurs in peaks around particular times of the day.

Daily biting peaks are dependent on species, season, climatic conditions, parity status (e.g., pars and nullipars seeking bloodmeals at different times of the day), location and host availability (Duke, 1968; Wenk, 1981; Crosskey, 1990). Since host-seeking periodicity is highly regulated by the duration of the gonotrophic (blood feeding and oviposition) cycle, biting activity of parous females may provide insights into the duration of such cycle (Cheke, 1995) and the timing of oviposition (Duke, 1968). Short gonotrophic cycles and blood

feeding soon after oviposition may translate into higher biting frequency and increased vectorial potential of the simuliid species (Collins et al., 1981). The interval between two consecutive bloodmeals forms part of the calculation of the vector biting rate on humans, a parameter which appears twice (squared) in the expressions for vectorial capacity and basic reproductive ratio of the parasite (Dietz, 1982; Basáñez and Boussinesq, 1999). Consequently, epidemiological models are sensitive to variations in this parameter (Kennedy and Basáñez, 2002; Kennedy et al., 2003).

The question of interest in this study was, therefore, whether or not there is a discernable periodicity in the diurnal host-seeking activity of the three main *O. volvulus* vectors in the Amazonian focus, an area where research has so far been sparse (but see Lacey and Charlwood, 1980; Narbaiza, 1986; Basáñez, 1992; Shelley et al., 1997). While Narbaiza (1986) and Basáñez (1992) worked mainly in the Sierra Parima highlands (≥ 250 m altitude), we focus here on localities situated below 250 m. Our work forms part of a larger investigation into entomological risk factors of human onchocerciasis, that together with parasitological (Botto et al., 1997, 1999; Vivas-Martínez et al., 2000a) and epidemiological (Vivas-Martínez et al., 1998, 2000b; Carabin et al., 2003) research aims at providing locale-specific data for parameterization of mathematical models for the population dynamics and control of the infection (Basáñez and Ricárdez-Esquinca, 2001).

2. Materials and methods

2.1. Study area

Descriptions of the study area and selection of fly-collection sites have been presented elsewhere (Grillet et al., 2001). Entomological sampling was carried out in five riverine Yanomami communities in southern Venezuela: Maweti-theri, Mahekoto-theri, Awei-theri, Hasupiwei-theri, and Pashopeka-theri, situated along the Ocamo–Putaco and Orinoco–Orinoquito river systems. The study villages were located between 140 m (Maweti) and 240 m (Pashopeka) above sea level and ranged in microfilarial prevalence between, respectively, 24 and 80% (Vivas-Martínez et al., 1998). The area is characterized by an average temperature

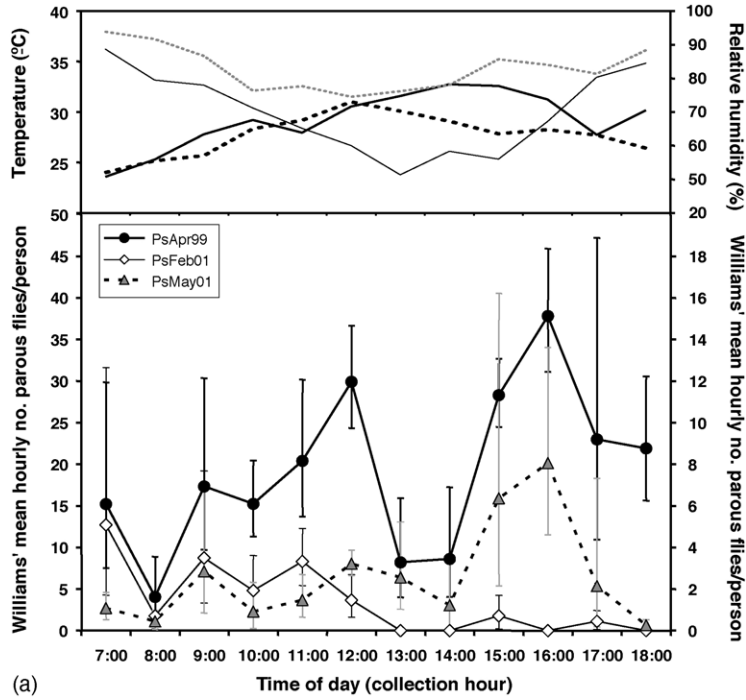
of 26–27 °C, lying in a very humid tropical forest (Huber et al., 1984). Annual rainfall (3750–5000 mm) is seasonally unimodal, with May to August being the rainiest months and October to March the driest. The transition between the dry and the wet season takes place around April, and that between the rainy and dry season around September (see Fig. 1 of Grillet et al., 2001).

2.2. Entomological methods

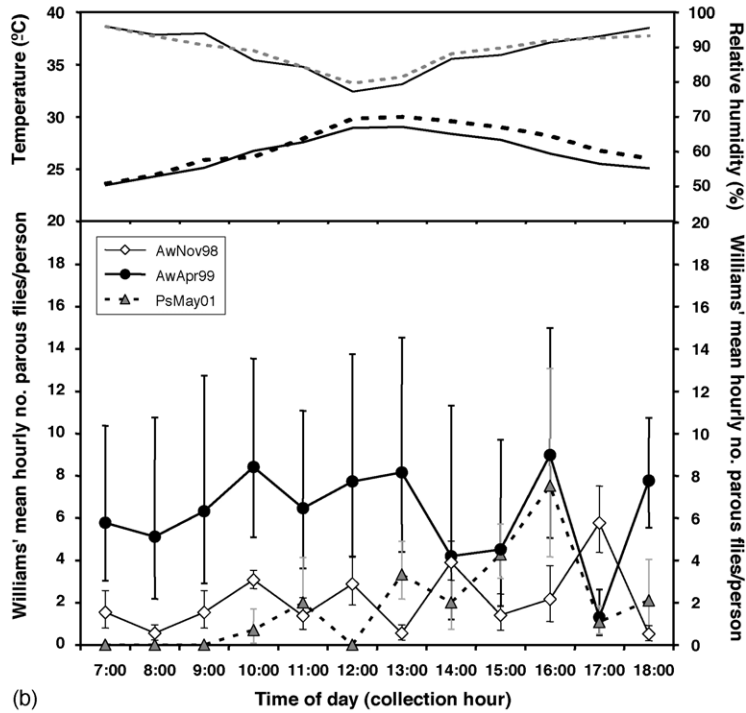
In each community, and during one to five consecutive days per visit, host-seeking flies that landed on two human attractants from the village were caught with manual aspirators by a team of two collectors (working during alternate hours) during the first 30 min of each hour, from 07:00 to 18:30 h. This amounted to 12 half-hour intervals or sampling units (hereafter referred to as the collection hour) per sampling day. Females were collected before procuring a blood-meal, thus what we refer to as biting rates are more truly landing rates. However, we will assume, for the purposes of this paper, that the number of flies that would have obtained a blood-meal (had they been allowed to do so) is proportional to the number of females alighting. Whenever possible, the collectors were the same throughout the study (NJV and JC) in order to minimize variations resulting from individual differences in catching ability. In the field, all hourly-caught flies were anaesthetized with chloroform vapour, identified to species, counted, and dissected for parity status according to criteria suggested by Wenk (1981). Taxonomic identification to morphospecies followed the keys by Ramírez-Pérez et al. (1982) and Shelley et al. (1997). Collections took place between 1995 and 2001, with communities visited more than once to cover both dry and rainy seasons (and their transitions) for each community. During each collecting day and site at each community, initial and final half-hourly readings of air temperature (°C) and relative humidity (RH, expressed in %) were conducted from 07:00 to 18:30 h with, respectively, a minimum–maximum mercury thermometer and a wet–dry bulb thermohygrometer.

2.3. Variables of interest

We focused mainly on the number of host-seeking parous flies per hour to describe diurnal biting period-



(a)



(b)

icity. Patterns of variation were analyzed both between i ($i = 1-12$) hours of a day and between j ($j = 1-n$) consecutive days ($n = 3-5$). However, and in order to obtain an overall picture per species, locality, and season, the average number of parous flies per collection hour (\bar{P}_i) was estimated using the geometric mean of Williams, which normalized the distribution of fly counts (Williams, 1937):

$$\bar{P}_i = \left[\prod_{j=1}^{j=n} (P_{i,j} + 1) \right]^{1/n} - 1 \quad (1)$$

The (asymmetrical) confidence intervals around the \bar{P}_i values were estimated according to Kirkwood and Sterne (2003).

2.4. Data analysis

Analyses were conducted according to species, locality and season. The sequence of observations, i.e., $\log(P_{i,j} + 1)$, was ordered along the (independent) time axis (hours i (from 07:00 to 18:00) within each of the consecutive j (from 1 to n) collection days and considered as a time series dataset (36–60 log-transformed observations per sampling visit). Assuming a cyclical trend in the data series (this is, a circadian cycle with period, $T = 24$ h), we aimed at identifying statistically significant maxima or biting peaks in the population of parous flies. To this end, we carried out a harmonic or periodic correlation analysis (Legendre and Legendre, 1998), which is well suited to the study of small (<100 observations) ecological data series. This method uses the first term of a Fourier series (Eq. (2)) to analyze ecological cyclic phenomena in which sinusoidal periodicity (e.g., circadian) may be expected. The explanatory variable x (=hours i of the day) is consequently transformed into a cyclic variable x' ,

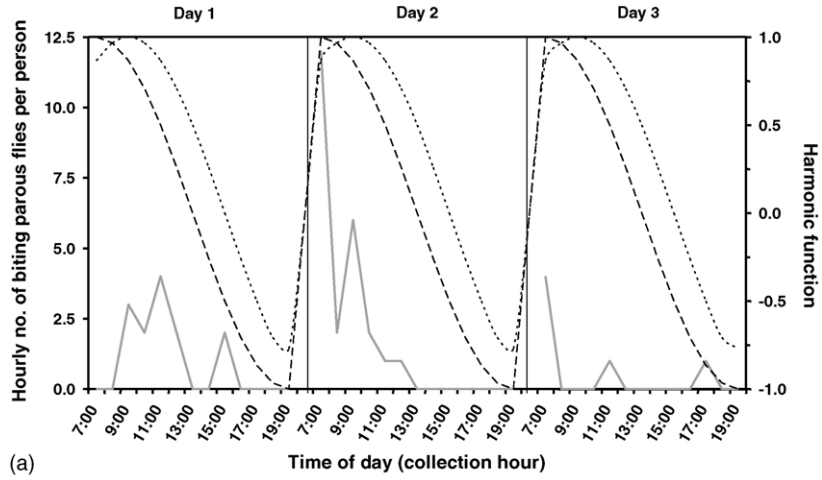
$$x' = \cos\left(\frac{2\pi(x \pm c)}{T}\right) \quad (2)$$

In Eq. (2), c is a constant that fits the position of the cosine along the abscissa, so that it corresponds to the time of minimum and maximum values in the dataset. When $c = 0$, for instance, the function peaks at 12:00 h. In order to detect the extent to which the original data series of $\log(P_{i,j} + 1)$ observations exhibited concordant periodic variation with the transformed x' variable, correlation analyses using Spearman's r were conducted between these both variables by shifting x' with respect to the number of parous flies, with time-lags of c units ($c = 1-6$). The positive sign in Eq. (2) shifts the series forwards (towards 18:00 h) and the negative sign shifts the series backwards (towards 06:00 h). The results thus obtained were plotted in a periodogram (Legendre and Legendre, 1998). Positively significant correlation(s) identifies the maximum (or maxima) biting peak(s) during the day. Hence, the purpose of the analysis is to identify which daylight hours best explain the variance observed in the response variable $y = \log(P_{i,j} + 1)$. However, and for graphical purposes only we show the (untransformed) hourly number of parous flies per person ($P_{i,j}$) versus collection hour and superimpose the best (most significant) periodic function(s) in the figures described in Section 3 of this report.

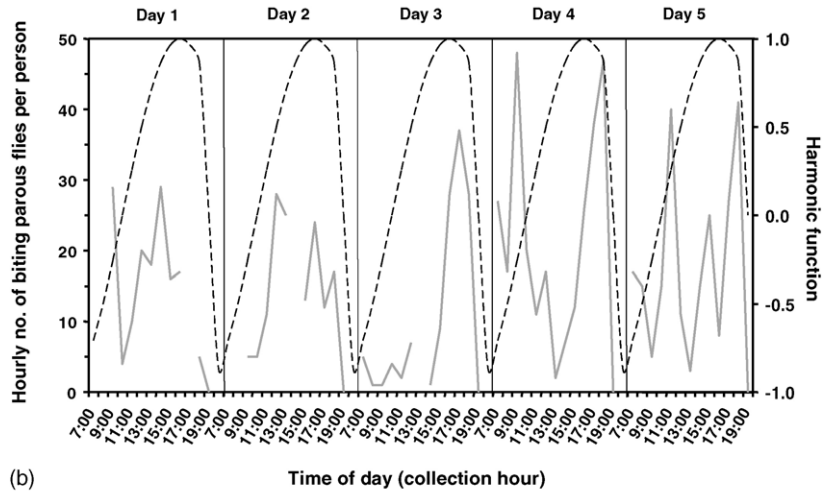
Separately, cross-correlation analyses were also conducted between the log-transformed numbers of parous flies and hourly air temperature and relative humidity values, shifting the series of the two latter variables with respect to y with time-lags of k -hours ($k = 0-2$) and using Spearman's r (Legendre and Legendre, 1998). Since lagging the series did not improve correlations, only $k = 0$ was used in subsequent analysis. Spatial (locality) and seasonal (sampling month) variations of the meteorological variables were explored by analysis of variance (one factor, fixed-effects ANOVA).

Finally, we explored the relationship between the response variable $y = \log(P_{i,j} + 1)$ and the explanatory variables: hour (as, a cyclic variable), hourly temperature (in °C), and relative humidity (in %) by using a

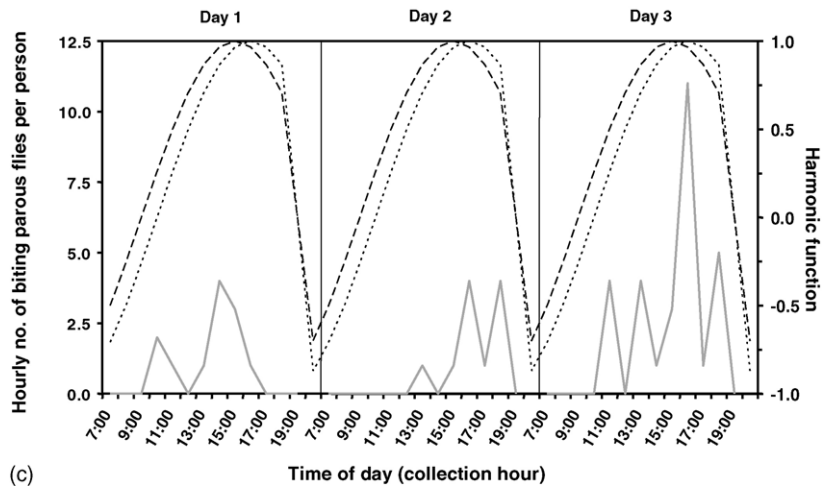
Fig. 1. Lower panels: Williams' mean-hourly number of parous flies per person (\bar{P}_i) \pm 95% confidence intervals vs. time of day (collection hour) for *Simulium guianense* s.l. in the Yanomami localities of (a) Pashopeka-theri (Ps) and (b) Awei-theri (Aw) in the Amazonian onchocerciasis focus of southern Venezuela. White diamonds and thin solid line: dry season (February 2001 in Pashopeka-theri and November 1998 in Awei-theri); black circles and thick solid line: dry-rainy transition period (April 1999); grey triangles and broken line: rainy season (May 2001). The right hand axis of (a) (on a scale of 0–20) refers to both the February and May 2001 data. Upper panels: average-hourly temperatures (°C; thicker lines on left axis) and relative humidity (%; thinner lines on right axis) for February 2001 (black solid lines) and May 2001 (grey broken lines) in (a) and for April 1999 (black solid lines) and May 2001 (grey broken lines) in (b).



(a)



(b)



(c)

multiple regression model:

$$y = \beta_0 + \beta_1 x' + \beta_2 \text{temp} + \beta_3 \text{RH} \quad (3)$$

where the hourly temperature (temp) and relative humidity (RH) are the averages of the half-hourly readings corresponding to each collection hour. All statistical analyses were conducted using STATISTICA™ (StatSoft, 1994) and they were considered significant at $P < 0.05$.

2.5. Ethical considerations and conflicts of interest

The leaders of the communities were informed of the objectives of the study prior to the commencement of collections and participants freely consented to act as blackfly attractants. The presence of entomological teams in the communities was followed shortly by medical teams who conducted parasitological evaluations and distributed mass ivermectin treatment. All fly attractants were therefore treated. There are no conflicts of interest to report.

3. Results

A total of 91,121 flies were caught during 118 sampling days between 1995 and 2001. Temperature and relative humidity were very similar from one day to the next for each sampling visit, but there was much variation within each day. (The results of ANOVA between mean temperature and hour, and between mean relative humidity and hour for all localities and sampling visits were, respectively, $F = 42.85$, d.f. = 11, $P < 0.001$ and $F = 21.35$, d.f. = 11, $P < 0.001$.) Within the day, temperature was correlated inversely with relative humidity, ranging from 23 °C and 96% at 07:00 h to 37 °C and 51% at 12:00 h ($r_S = -0.84$, $P < 0.05$, $n = 276$; all samples, localities, months, and hours). In general, maximum temperature (and minimum humidity) values were reached between 11:00 and 15:00 h (upper panels of Figs. 1, 3, and 5).

3.1. *Simulium guianense* s.l.

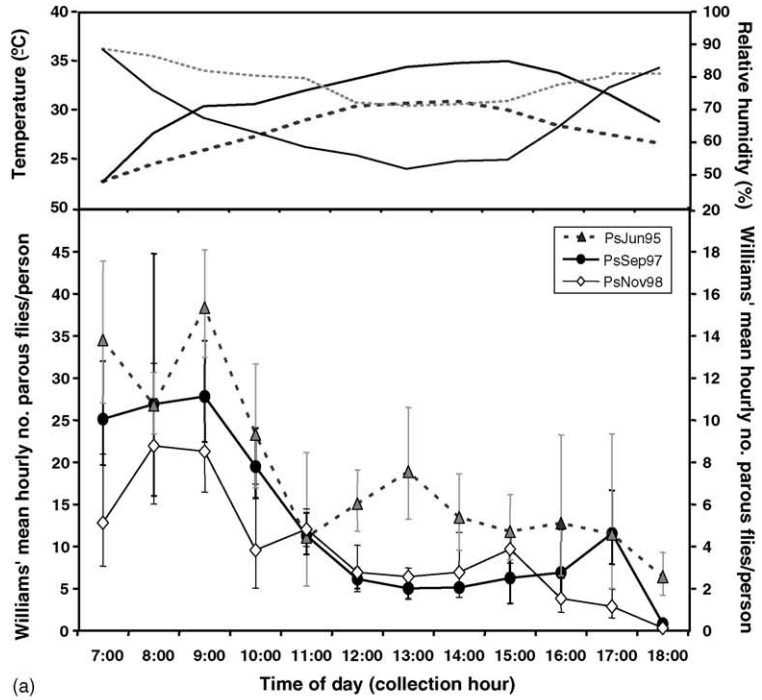
This species prevailed in the villages of Pashopeka- and Awei-theri particularly during the dry–rainy transition (April), being more abundant in the former (Fig. 1a) than in the latter (Fig. 1b) community. Although also collected in Hasupiwei- and Mahekoto-theri, its very low numbers in these villages precluded any analysis for these localities. *S. guianense* was virtually absent in Maweti-theri. Overall, the host-seeking behaviour of parous females showed a bimodal activity at variable times of the day depending mainly on season and to a lesser extent on locality (Fig. 1).

In the transition between the dry and rainy seasons, when the mean fly density reached its highest levels (up to 38 parous flies/person-hour at Pashopeka in April 1999; Fig. 1a), parous *S. guianense* s.l. bit during every daylight hour, with two distinct maxima of activity (a lower peak in the morning, and a higher peak between 15:00 and 17:00 h). According to the harmonic analysis, the afternoon biting peak showed a statistically significant correlation with 16:00 h at Pashopeka ($r_S = 0.30$, $n = 54$, $P < 0.01$; Fig. 2b).

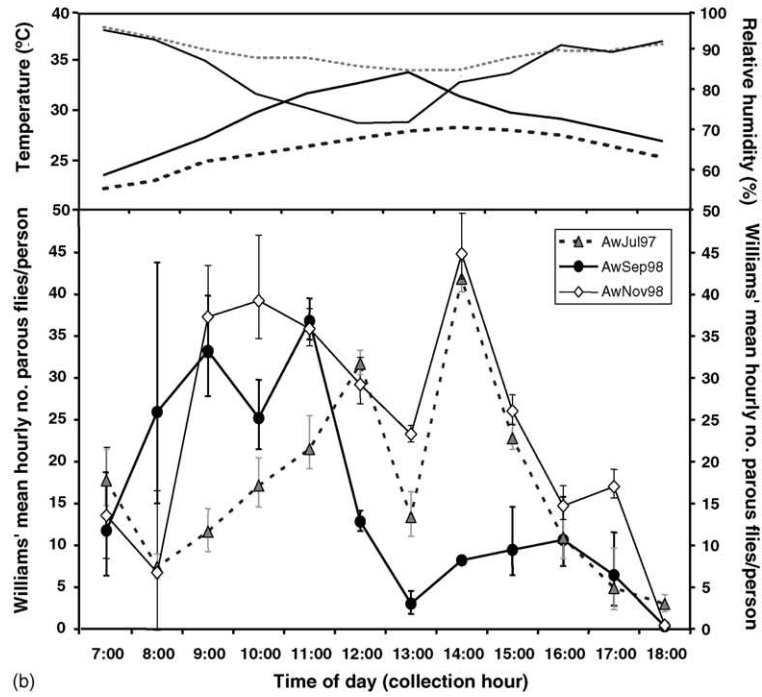
In the rainy season (May 2001 in Fig. 1a for Pashopeka- and Fig. 1b for Awei-theri), there was a considerable reduction of morning parous activity, whereas the afternoon peak remained the highest and, again, showed a statistically significant correlation with 16:00 h at Awei-theri ($r_S = 0.61$, $n = 36$, $P < 0.01$; Fig. 2c). By the middle of the dry season (February 2001) parous activity became mainly matinal, with significant peaks at 07:00 and 09:00 h at Pashopeka ($r_S = 0.50$ for both peaks; $n = 36$; $P < 0.05$; Fig. 2a). By contrast, at Awei-theri activity always peaked during late afternoon (17:00 h) throughout the whole of the dry season (November 1998; Fig. 1b).

Relative humidity was higher and temperature was lower at Awei-theri than at (Pashopeka) despite the fact that Pashopeka (240 m) is located at a higher altitude than Awei-theri (162 m). (There were significant differences between the five localities with respect to temperature: $F = 9.26$, d.f. = 4, $P < 0.001$, and

Fig. 2. Time series of the hourly number of parous *S. guianense* s.l. flies coming to feed on human attractant (solid grey lines on left axis) for each of the collection days in a sampling period, and amplitude of harmonic function(s) (broken lines on right axis) that best explain variation in parous fly density at (a) Pashopeka-theri, dry season (February 2001); harmonic function peaks = 07:00 (dashed line) and 09:00 (dotted line). (b) Pashopeka-theri, dry–rainy transition (April 1999); harmonic function peak = 16:00 (dashed line). (c) Awei-theri, rainy season (May 2001); harmonic function peak = 15:00 (dashed line) and 16:00 (dotted line).



(a)



(b)

Table 1

Results of the multiple regression model (Eq. (3)) between the log-transformed number of parous flies per hour ($y = \log(P_{i,j} + 1)$) and the explanatory variables of hour (as a cyclic variable, x'), hourly temperature (temp), and relative humidity (RH)

Species	Locality (date)	Best predictor variable	Coefficient ($\beta_i \pm \text{S.E.}$)	t	d.f.	R^2	P value
<i>S. guianense</i>	Ps (February 2001)	x' (07:00 h)	$\beta_1 = 0.52 \pm 0.06$	3.6	35	0.27	<0.001
<i>S. guianense</i>	Aw (May 2001)	x' (16:00 h)	$\beta_1 = 0.58 \pm 0.07$	4.1	35	0.33	<0.001
<i>S. incrustatum</i>	Ps (January 1997)	x' (13:00 h)	$\beta_1 = 0.32 \pm 0.28$	2.5	53	0.11	<0.05
<i>S. incrustatum</i>	Ps (June 1995)	x' (07:00 h)	$\beta_i = 0.40 \pm 0.07$	3.3	58	0.16	<0.05
<i>S. incrustatum</i>	Ps (September 1997)	x' (07:00 h)	$\beta_1 = 0.57 \pm 0.07$	5.3	59	0.40	<0.001
<i>S. incrustatum</i>	Aw (April 1999)	RH	$\beta_3 = -0.74 \pm 1.73$	-5.0	59	0.31	<0.05
<i>S. incrustatum</i>	Aw (May 2001)	x' (12:00 h)	$\beta_1 = 0.65 \pm 0.14$	4.9	35	0.42	<0.001

Only the results for the significant coefficients are shown (Ps: Pashopeka-theri; Aw: Awei-theri).

relative humidity: $F = 2.93$, d.f. = 4, $P < 0.05$, respectively.) There was more between-season variation in these variables at Pashopeka than at Awei-theri (upper panels of Fig. 1a and b, respectively). During the rainy season of May 2001, the biting activity of *S. guianense* was positively associated with increasing temperature at both Pashopeka ($r_S = 0.41$, $n = 36$, $P < 0.05$) and Awei-theri ($r_S = 0.42$, $n = 36$, $P < 0.05$), with warmer hours (25–29 °C) promoting more biting activity than cooler ones (<25 °C), and negatively related with increasing humidity ($r_S = -0.35$, $n = 36$, $P < 0.05$ and $r_S = -0.24$, $n = 36$, $P < 0.05$, respectively), with a very wet time, i.e., >90% RH reducing fly activity (upper panels of Fig. 1a and b). The opposite was observed at Pashopeka during February 2001 (dry season), when correlations of parous flies were negative with temperature ($r_S = -0.41$, $n = 36$, $P < 0.05$) and positive with RH ($r_S = 0.45$, $n = 36$, $P < 0.05$). The high temperatures (>30 °C) and low humidity values (<60%) reached between midday and early afternoon, presumably, concentrated parous biting activity during morning hours (February 2001; Figs. 1a and 2a). In Awei-theri, and during the dry–rainy transition of April 1999, biting activity tracked temperature ($r_S = 0.33$, $n = 60$, $P < 0.01$) and was inversely related with humidity ($r_S = -0.39$, $n = 60$, $P < 0.01$) (Fig. 1b).

In those localities or sampling visits in which the biting behaviour of parous *S. guianense* was simultaneously and significantly explained by the three ex-

planatory variables of hour, temperature, and humidity, most of the variation was explained by hour rather than by changes in the remaining two covariates as shown by the multiple regression results summarized in Table 1.

3.2. *Simulium incrustatum*

This species was collected solely at Pashopeka- and Awei-theri, being less abundant in the former (maximum mean-hourly density of 28 parous flies/person) than in the latter (45 parous flies/person). At Pashopeka vector density was higher during the rainy–dry transition (September 1997) and dry (November 1998) seasons than during the rains (June 1995) (Fig. 3a). At Awei-theri, by contrast, parous biting activity was less intense during the rainy–dry transition (September 1998) than during the dry (November 1998) season (Fig. 3b). However, it must be stressed that these comparisons are made between snapshots taken during different years, and that perhaps more importantly are the consistent patterns of activity that emerge across the years. In this respect, correlation and harmonic analyses indicated the existence of a primarily unimodal pattern of diurnal activity for parous *S. incrustatum*, with the timing of maximum biting showing variability according to locality and to a lesser extent according to season. At Pashopeka, most biting activity was concentrated in early morning (07:00–09:00 h) significant

Fig. 3. Lower panels: Williams' mean-hourly number of parous flies per person (\bar{P}_i) \pm 95% confidence intervals vs. time of day (collection hour) for *Simulium incrustatum* in the Yanomami localities of (a) Pashopeka-theri (Ps) and (b) Awei-theri (Aw). White diamonds and thin solid line: dry season (November 1998); grey triangles and broken line: rainy season (June 1995 in (a) and July 1997 in (b)); black circles and thick solid line: rainy–dry transition (September 1997 in (a) and September 1998 in (b)). The right hand axis of (a) (on a scale of 0–20) refers to the June 1995 data. Upper panels: average-hourly temperatures (°C; thicker lines on left axis) and relative humidity (%; thinner lines on right axis) for June 1995 (grey broken lines) and September 1997 (black solid lines) in (a) and for July 1997 (grey broken lines) and September 1998 (black solid lines) in (b).

peaks ($r_S = 0.60$, $n = 60$, $P < 0.01$ for both 07:00 and 08:00 peaks, September 1997, and $r_S = 0.40$, $n = 48$, $P < 0.01$ for peak at 07:00, September 1998; Fig. 4a; $r_S = 0.56$, $n = 57$, $P < 0.01$ for both 08:00 and 09:00 peaks, November 1998; Fig. 4b). During these months (rainy–dry and dry seasons), parous fly activity was negatively associated with air temperature ($r_S = -0.35$, $n = 60$, $P < 0.01$) and positively associated with relative humidity ($r_S = 0.27$, $n = 60$, $P < 0.05$) (upper panel

in Fig. 3a). This was particularly true in September 1997, when high temperatures ($>30^\circ\text{C}$) and low humidity ($<60\%$) values were reached during midday and early afternoon. However, in January 1997 and May 2001, when early morning temperatures were as low as $23\text{--}24^\circ\text{C}$, and relative humidity as high as 94%, the biting peak shifted towards midday (data not shown). In fact, biting activity was occasionally significantly explained by changes in air temperature and relative

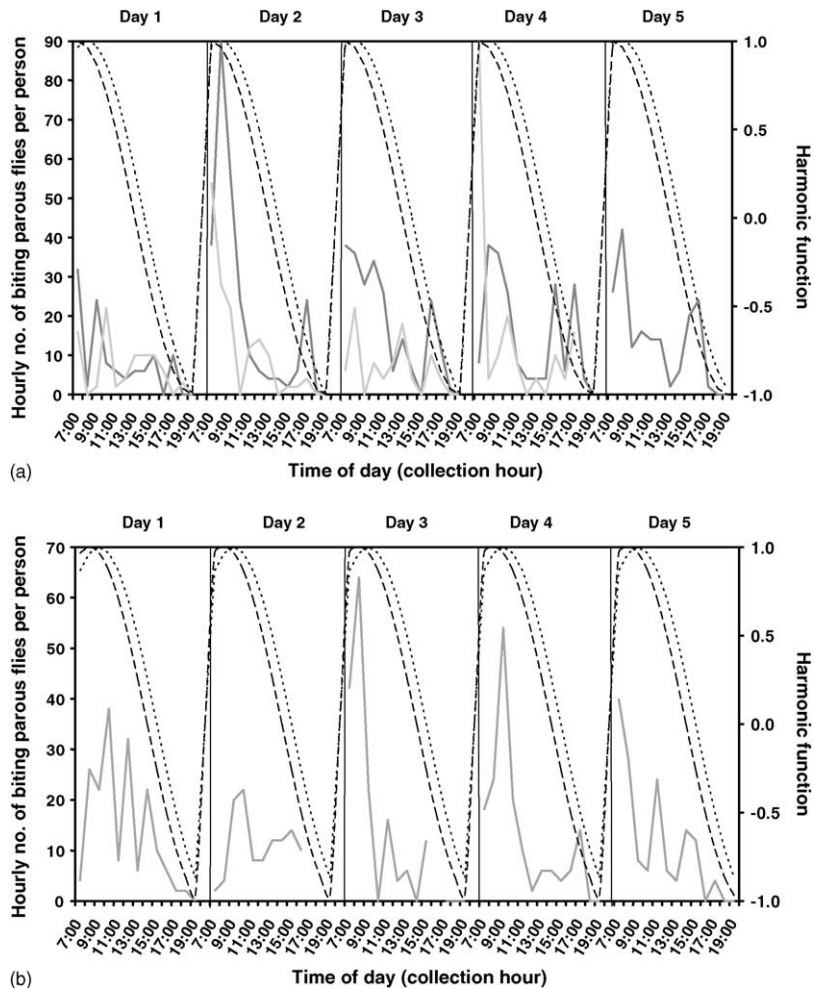


Fig. 4. Time series of the hourly number of host-seeking parous *S. incrustatum* flies (solid grey lines on left axis) for each of the collection days in a sampling period, and amplitude of harmonic function(s) (broken lines on right axis) that best explain variation in parous fly density at (a) Pashopeka-theri, rainy–dry transition; September 1997: dark grey and September 1998: light grey; harmonic function peaks = 07:00 (dashed line) and 08:00 (dotted line); (b) Pashopeka-theri, dry season (November 1998); harmonic function peaks = 08:00 (dashed line) and 09:00 (dotted line); (c) Awei-theri, rainy season (July 1997); harmonic function peaks = 12:00 (dashed line), 13:00 (dotted line) and 14:00 (dot-dash line); (d) Awei-theri, rainy–dry transition (September 1998); harmonic function peaks = 09:00 (dashed line) and 10:00 (dotted line).

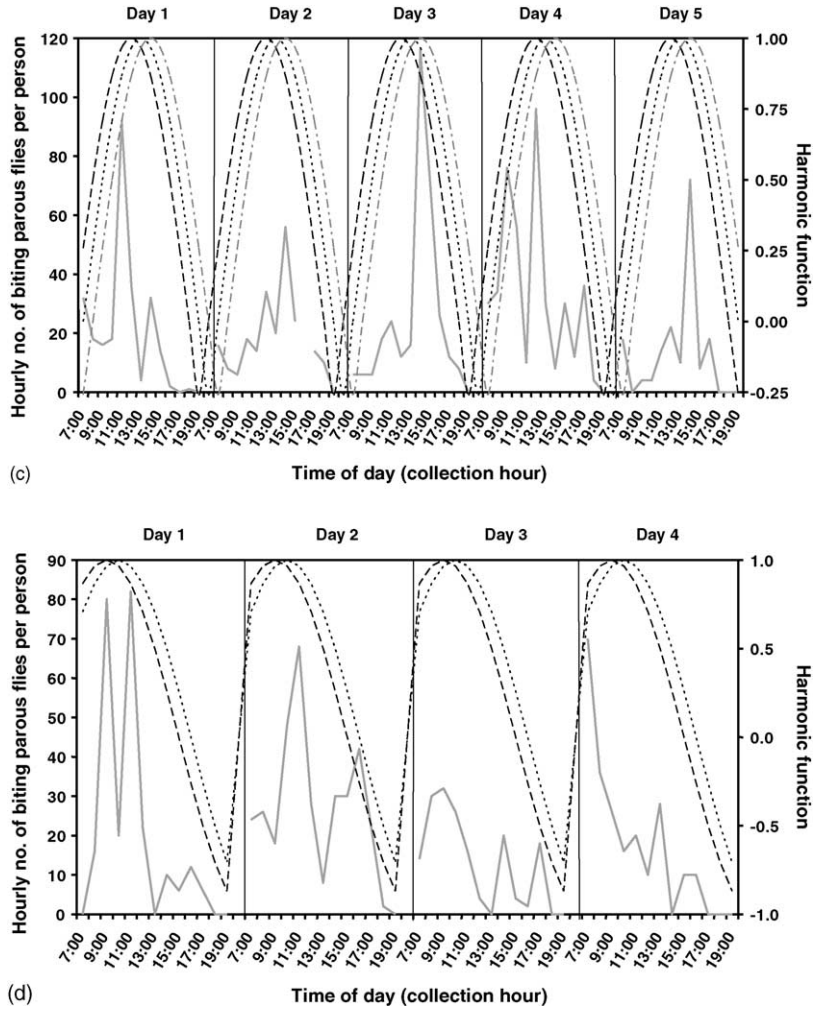


Fig. 4. (Continued).

humidity. For instance, early morning or late afternoon lower temperatures and wetter periods were associated with reduced parous activity during those periods in the dry–rainy (April) and rainy (July) seasons at Awei-theri (July 1997; Fig. 3b), or during the dry season at Pashopeka (November 1998; Fig. 3a).

In Awei-theri, and during the rains biting density of parous females peaked between 12:00 and 15:00 h (Fig. 3b), and this was corroborated by correlation and harmonic analyses ($r_S = 0.36$, $r_S = 0.33$ and $r_S = 0.28$, $n = 59$, $P < 0.05$ for, respectively, maxima at 12:00, 13:00, and 14:00 h, July 1997; Fig. 4c), although there

was a small lull in biting around noon. During September 1998 in particular, when midday temperatures and relative humidity were, respectively, as high as 34°C and as low as 70%, peak biting activity shifted towards mid-morning (08:00–11:00 h; Fig. 3b), and this was also highlighted by statistically significant peaks taking place at 09:00 ($r_S = 0.56$, $n = 48$, $P < 0.01$) and 10:00 ($r_S = 0.54$, $n = 48$, $P < 0.01$) hours in the harmonic analysis (Fig. 4d). In general, however, the biting behaviour of parous *S. incrustatum* was best explained by hour than by daily changes in temperature and/or relative humidity (Table 1).

3.3. *Simulium oyapockense* s.l.

This species occurred mainly at Maweti-theri (with an average of up to 420 parous flies/person-hour in May 1995), followed by Mahekoto-theri (260 parous flies/person-hour in April 1999) and Hasupiwei-theri (140 parous flies/person-hour in April 1999) (Fig. 5a–c, respectively). In Pashopeka- and Awei-theri this species was less abundant but high parous fly densities were seen during the dry season (February) of 2001 (350 and 180 parous flies/person-hour in, respectively, Pashopeka- and Awei-theri).

Parous *S. oyapockense* s.l. displayed quite different biting patterns from those of *S. guianense* and *S. incrustatum*, with highly variable biting activity according to locality and sampling periods (Fig. 5). At Maweti-theri (Fig. 5a), parous flies showed a relatively constant activity throughout the day, perhaps more pronounced between 10:00 and 16:00 h, but without any prominent peak being picked up by the harmonic analysis. In January 1997, a more clearly bimodal pattern was observed, with an early morning peak (starting at 07:00 h), and a second (minor) peak between 16:00 and 17:00 h (data not shown). Similarly, a relatively

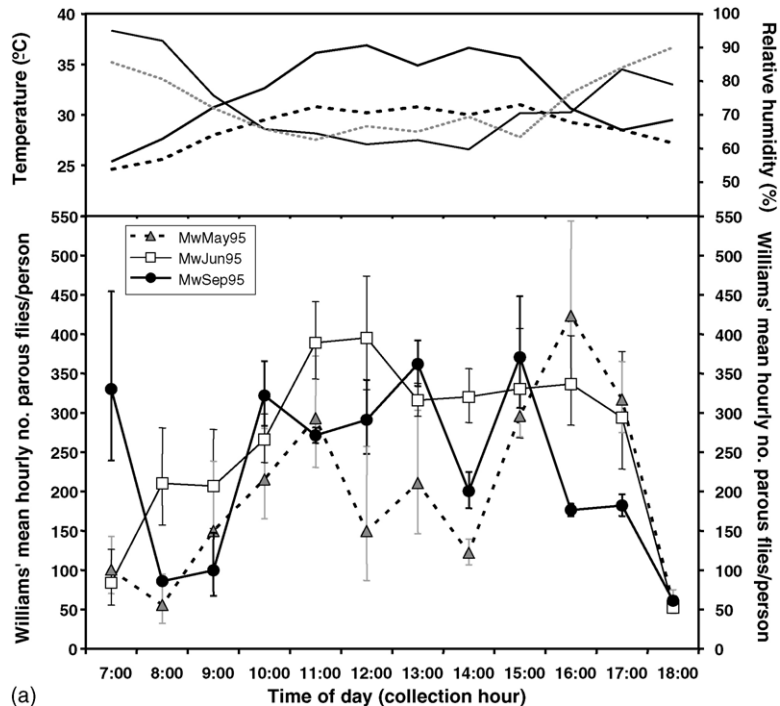


Fig. 5. Lower panels: Williams' mean-hourly number of parous flies per person (\bar{P}_i) \pm 95% confidence intervals vs. time of day (collection hour) for *Simulium oyapockense* s.l. in the Yanomami localities of (a) Maweti-theri (Mw) in the rainy seasons of May (grey triangles and broken line) and June (white squares and thin solid line), and the rainy–dry transition of September (black circles and thick line) 1995; (b) Mahekoto-theri (Mh) in the dry season of February 1997 (grey diamonds and broken line); the dry–rainy transition of April 1999 (white circles and black thin line), and the rainy–dry transition of September 1997 (black circles and thick line on the right hand scale of 0–200); (c) Hasupiwei-theri (Hs) in the dry season of February 1997 (white diamonds and black thin line); dry–rainy transition of April 1999 (black circles and thick line), and rainy season of August 1997 (grey triangles and broken line); (d) Pashopeka-theri (Ps) and (e) Awei-theri (Aw) in the rainy–dry transition of September 1998 (black circles and thick line) and the dry seasons of November 1998 (white diamonds and thin black line) and February 2001 (grey diamonds and broken line). In (d) and (e) the data for September 1998 are plotted on the right hand scale of 0–100. In (d) the data for November 1998 are also plotted on the right axis. Upper panels: average-hourly temperatures ($^{\circ}$ C; thicker lines on left axis) and relative humidity (%) (thinner lines on right axis) for May (broken lines) and September (solid lines) 1995 in (a); February (broken lines) and September (solid lines) 1997 in (b); August 1997 (broken lines) and April 1999 (solid lines) in (c); and September 1998 (solid lines) and February 2001 (broken lines) in (d) and (e).

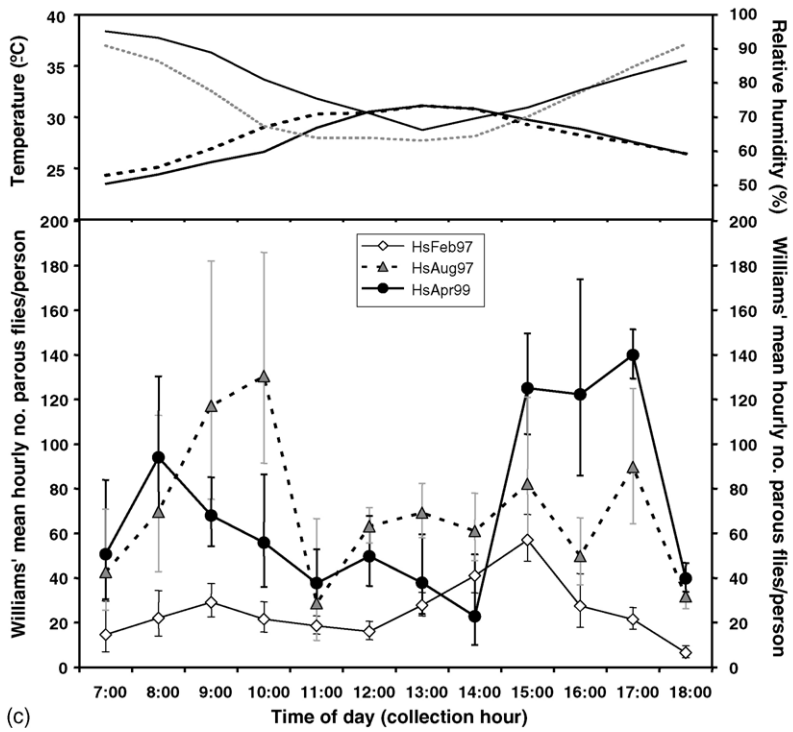
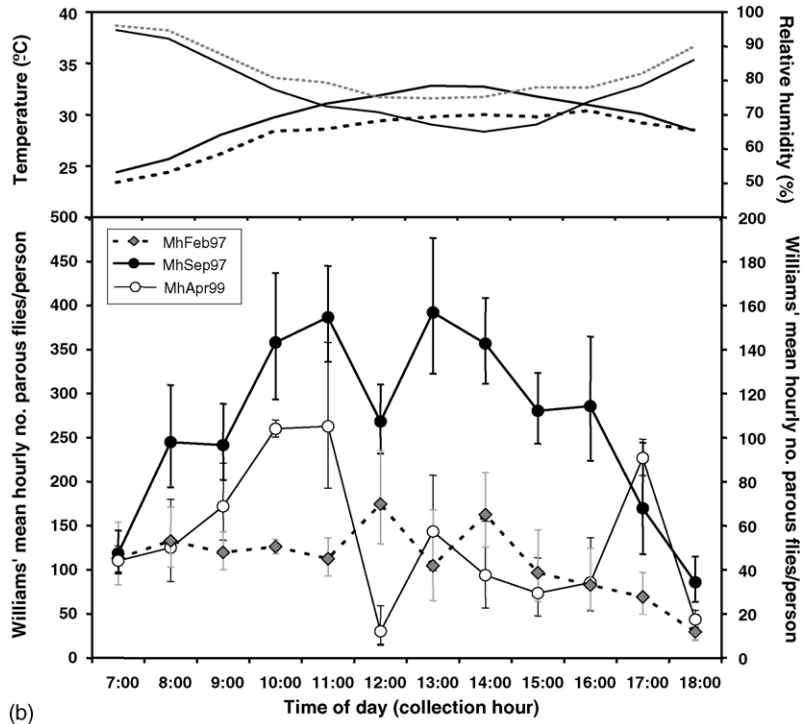


Fig. 5. (Continued).

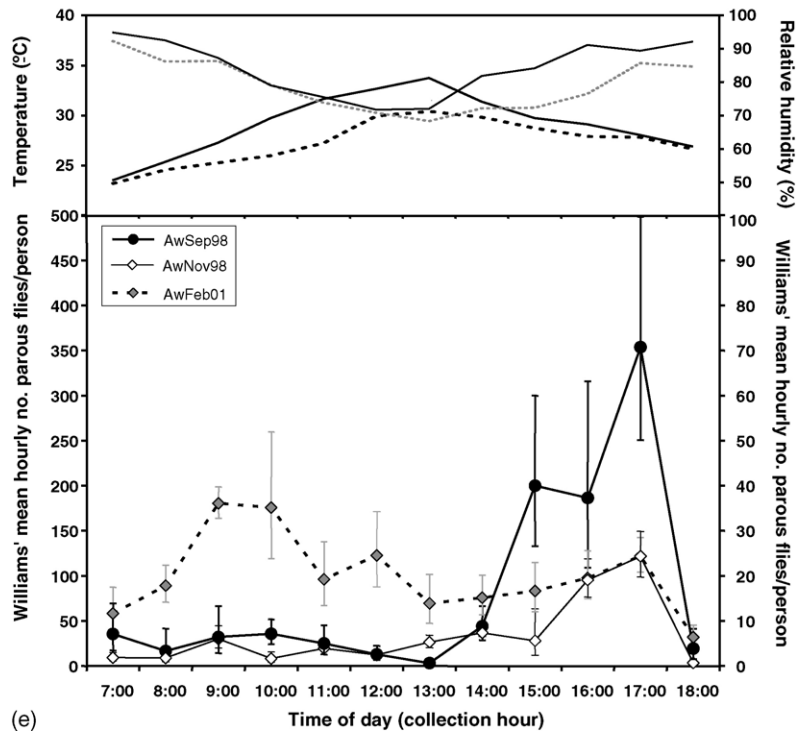
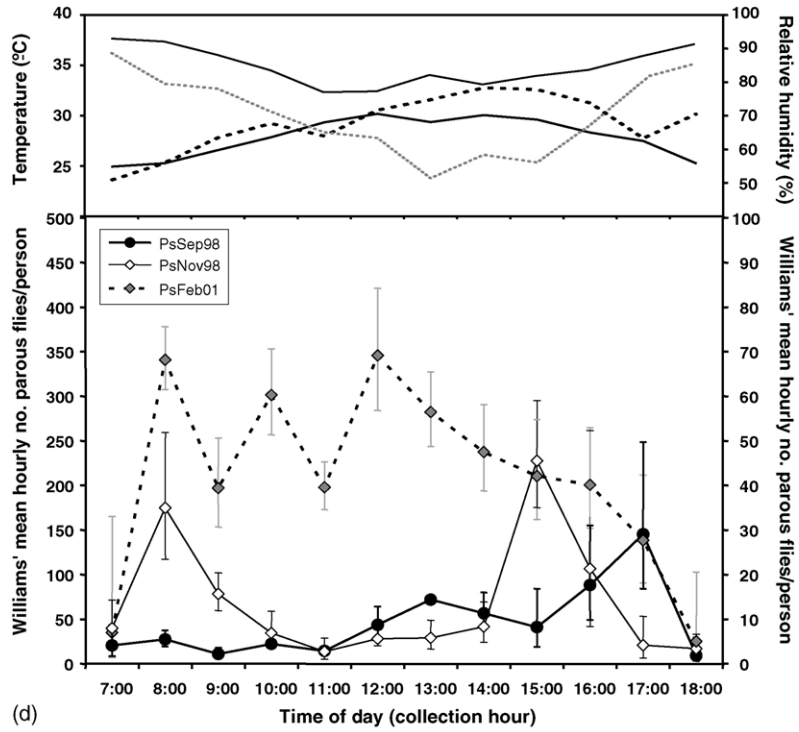


Fig. 5. (Continued).

constant pattern of activity was observed throughout the day during the dry season and rainy–dry transition (respectively February and September 1997) at Mahekoto-theri (Fig. 5b). The pattern observed during the dry–rainy transition of April 1999 tends to echo that of the rainy–dry transition (Fig. 5b). At Hasupiwei-theri, parous fly activity showed a more bimodal pattern, with biting peaks during the morning (08:00–11:00) and afternoon (15:00–17:00) hours (Fig. 5c). However, none of the Spearman correlations

in the harmonic analysis were statistically significant for Hasupiwei.

In Pashopeka, where the parous fly density of *S. oyapockense* was low by comparison to that of the lower altitude localities of Maweti-, Mahekoto-, and Hasupiwei-theri (<200 m), parous biting activity intensified in the afternoon, peaking between 15:00 and 18:00 h (Fig. 5d) with a significant maximum detected at 17:00 h ($r_s = 0.43$, $n = 46$, $P < 0.01$, September 1998; Fig. 6a). By contrast, and during February

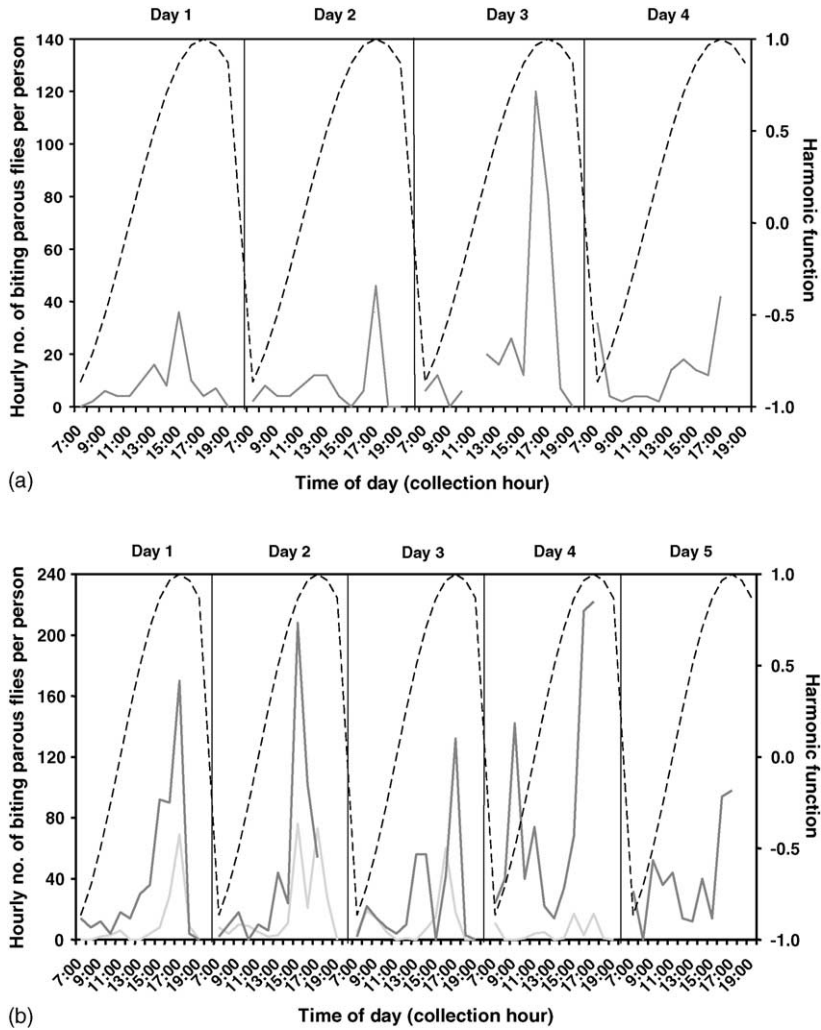


Fig. 6. Time series of the hourly number of parous *S. oyapockense* s.l. flies caught on human host (solid lines on left axis) for each of the collection days in a sampling period, and amplitude of harmonic function(s) (broken lines on right axis) that best explain variation in parous fly density variation at (a): Pashopeka-theri, rainy–dry transition (September 1998); harmonic function peak = 17:00 (dashed line); (b): Awei-theri, rainy–dry transition (September 1998: light grey) and dry season (November 1998: dark grey); harmonic function peak = 17:00 (dashed line).

2001, when the density of parous *S. oyapockense* at this locality was unusually high, parous biting activity was concentrated in the morning (from 08:00 to 12:00) followed by a steady decline during the afternoon (Fig. 5d). Finally, the dominant trend in the host-seeking parous population at Awei-theri was primarily unimodal, with parous females predominantly biting between 15:00 and 18:00 h during the more typical densities of September and November 1998 (Fig. 5e) with a significant peak again at 17:00 h ($r_S = 0.41$, $n = 48$, $P < 0.01$, September 1998, and $r_S = 0.50$, $n = 57$, $P < 0.01$, November 1998; Fig. 6b), or shifting towards the morning (between 08:00 and 11:00 h) during the unusually high fly densities recorded in February 2001, with a minor (non-significant) peak at 17:00 h (Fig. 5e).

Mostly, host-seeking behaviour of *S. oyapockense* in relation to the recorded meteorological variables tended to be positively correlated with air temperature and inversely correlated with relative humidity in Maweti (respectively, $r_S = 0.35$ and $r_S = -0.24$, $n = 53$, $P < 0.05$ and $P = 0.07$ in May 1995; $r_S = 0.42$ and $r_S = -0.47$, $n = 22$, $P < 0.05$ in September 1995; Fig. 5a); Mahekoto ($r_S = 0.34$ and $r_S = -0.30$, $n = 60$, $P < 0.01$ in September 1997; $r_S = -0.36$ (RH), $n = 60$, $P < 0.01$ in February 1997; Fig. 5b); Hasupiwei-theri ($r_S = -0.39$ (RH), $n = 54$, $P < 0.01$ in August 1997; Fig. 5c); Pashopeka ($r_S = 0.33$ (temperature), $n = 46$, $P < 0.05$ in September 1998; Fig. 5d), and Awei-theri ($r_S = 0.42$ and $r_S = -0.56$, $n = 36$, $P < 0.01$ in February 2001; Fig. 5e). In particular during the rains, warmer (and drier) hours promoted more biting than cooler (<24 °C) and wetter periods (>90% RH). In none of the localities was the biting behaviour of *S. oyapockense* s.l. simultaneously explained by hour, temperature and relative humidity.

4. Discussion

In the Amazonian onchocerciasis focus of southern Venezuela, it can be said that the biting periodicity of the three vector species under investigation is strongly species-specific but influenced, to a lesser degree, by locality, season, and daily changes in weather conditions. The parous females of *S. guianense* s.l. showed a predominantly bimodal activity pattern, biting mainly during mid-morning and more prominently, mid-afternoon. *S. incrustatum* exhibited mainly uni-

modal activity, with biting peaks during the morning or, under some circumstances, at midday. *S. oyapockense* s.l., on the contrary, bit throughout the day, mainly between 10:00 and 16:00 h (although bimodal periodicity was sometimes observed). These results are in broad agreement with those of previous studies (Lacey and Charlwood, 1980; Narbaiza, 1986; Shelley et al., 1997).

4.1. The role of endogenous, species-specific cycles

Species-specific hourly biting patterns were generally consistent throughout the study period in spite of seasonal and yearly variations, as hour of the day was by and large the best predictor of parous female biting activity at each of the study localities. This suggests that host-seeking periodicity in these simuliid species is largely determined by endogenous rhythms probably driven by blood feeding and oviposition cycles. Dalmat (1955), working with Guatemalan blackflies (*S. ochraceum*, *S. metallicum*, *S. callidum*) and Alverson and Noblet (1976), investigating North American simuliids (*S. slossonae* among others) found that time of the day was the variable best explaining observed patterns of host-seeking activity in such blackflies, and suggested the operation of circadian control mechanisms. Similar arguments have been proposed by Lacey and Charlwood (1980) for Brazilian simuliids including *S. guianense* s.l. However, it is clear that biting periodicity is also readily modifiable (promoted or suppressed) by environmental (favourable or unfavourable) factors that may influence host location, feeding or egg-laying, helping to explain some departures from otherwise well-defined fly activity patterns.

4.2. The role of overall fly abundance

For each species and locality, daily variation in the level of host-seeking activity may be caused by changes in overall fly abundance. Adult fly abundance is influenced, among other factors, by rates of emergence and mortality, which may vary seasonally. For instance, locally unsuitable breeding sites due to seasonal variation might affect negatively the rate of recruitment to the adult population which, in turn, might influence the level of biting. We have already shown that seasonal variation of parous biting rates of *S. incrustatum* and

S. oyapockense s.l. are strongly correlated with river levels and breeding site dynamics in the study localities (Grillet et al., 2001). In this report, *S. guianense* s.l. and *S. oyapockense* s.l. showed a greater degree of seasonal variation in biting pattern than *S. incrustatum*, which could be explained in part by daily and seasonal variations in the rates of recruitment to the biting population. It is expected that as fly density reaches its maximum, observed periodicity will increasingly reflect true underlying host-seeking patterns as the rhythms of individual flies will sink in phase (or in synchrony) with waves of communal emergence and oviposition at population level (Corbet, 1966). In *S. guianense* s.l., the greatest degree of variation in the timing of biting peaks was observed at Awei-theri, where the species showed lower biting parous rates than at Pashopeka (Fig. 1b). The same applied to *S. oyapockense* s.l. at Hasupiwei-theri (Fig. 5c). Our observations resemble those of Renz (1987) on *S. damnosum* s.l. in northern Cameroon.

4.3. The role of breeding and resting sites

Between-locality variation in the diurnal host-seeking patterns of parous flies could also be attributed to variations in the proximity of breeding (ovipositing) sites, resting (post-prandial) sites, blood host populations, and dispersal ability of parous flies. For instance, *S. incrustatum* showed variation in the timing of its morning biting peaks between Pashopeka- and Awei-theri, with the former locality having a consistently earlier peak of parous *S. incrustatum* activity than the latter, where the peak occurred later in the morning perhaps indicating that suitably oviposition sites are further away from the village in Awei-theri than in Pashopeka for this particular species. This would contrast with a situation in which female flies could arrive from many different, scattered, and suitable breeding or resting sites, producing a more steady and uniform flow of host-seeking flies such as in the case of *S. oyapockense* s.l. at Maweti- and Mahekoto-theri. In addition, variation in host-seeking patterns between sibling members of simuliid species complexes has been observed within several Neotropical species (Shelley, 1988). Hence, an alternative explanation for the variability observed in *S. oyapockense* s.l. at Maweti-, Mahekoto- and Hasupiwei-theri (lowland areas) as opposed to Awei-theri and Pashopeka (higher altitude ar-

eas) is that of differences in cytospecies composition at these localities.

4.4. The role of microclimatic conditions

Changing meteorological conditions (at local level) are also an important source of biting variation. Diurnal and locality-specific variations in tropical simuliid man-biting density have been associated mainly to variations in air temperature and relative humidity (Dalmat, 1955; Collins, 1979; Lacey and Charlwood, 1980). In practice, it is difficult to disentangle the possibly separate effect of each of these variables due to their close and inverse relationship. In this study, we found that, for *S. guianense* and *S. incrustatum*, parous biting activity was, in general, promoted by warmer and drier hours during the rainy months, whereas the opposite relationship was observed during the drier and hotter months. Variation in the parous biting activity of *S. oyapockense* tended to track that of temperature and be negatively correlated with that in relative humidity in all localities and seasons. Although we observed biting activity up to 34 °C and 51% RH, the temperatures that on average promoted most biting ranged from 24 to 29 °C, with accompanying humidity ranging from 70 to 92%. When these factors were outside of these optimal ranges they had just the opposite effect, i.e., when temperature and relative humidity were much higher or lower than their typical values at each locality, a reduction or modification in the host-seeking activity was observed. This could help explain observed variations around a well-defined biting pattern (as with *S. guianense*), oscillations around a biting peak (*S. incrustatum*), or shifts and variability in the time of day when biting activity peaked (*S. oyapockense*). A more steady level of biting activity, as that observed in *S. oyapockense* s.l. at Maweti could be the result of fairly constant values of temperature and relative humidity throughout the day in lowland forested areas in contrast with the more pronounced variations registered at the higher altitude localities of Pashopeka- and Awei-theri. In a comparison of vector bionomics in forested versus open savanna localities of West Africa, Crosskey (1990) pointed out that biting activity levels of *S. damnosum* s.l. tend to be more stable in the former (where temperature, humidity and shade remain fairly constant throughout the day) than in the latter (with little shade). In more open settings the biting activity of savanna members of

the *damnosum* complex usually decreases at midday as temperature and humidity reach their highest and lowest levels, respectively, although unimodal activity is sometimes observed (Crosskey, 1955). In summary, subtle variations in microclimatic conditions among localities, as well as the between-season variation within localities could explain a great deal of the observed variation in blackfly biting patterns.

4.5. Implications for the timing of blackfly oviposition

Our results suggest that gravid *S. guianense* s.l. females may oviposit either early in the afternoon or perhaps around noon as indicated by the main biting peak of parous flies in mid-afternoon. Another oviposition cycle would occur during late evening (sunset) or very early in the morning (sunrise), with a subsequent peak of parous host-seeking activity in mid-morning. Most (gonotrophically concordant) blackfly species seek their next blood meal within a few (2–4) hours after laying eggs, with the preferred time for oviposition being that around sunrise or sunset (Crosskey, 1990). Blood feeding soon after emergence and oviposition has been suggested to increase the vectorial potential of otherwise relatively poor insect hosts for *O. volvulus* such as *S. ochraceum* s.l. in Guatemala (Collins et al., 1981). Patterns of emergence and oviposition are poorly known for the vectors in the Amazonian focus and deserve further investigation, particularly in the case of species as epidemiologically important as *S. guianense* s.l. An early morning oviposition (around sunrise) is inferred for *S. incrustatum* from its mainly unimodal and early to mid-morning peak of parous biting activity.

4.6. Epidemiological implications

Our results suggest that mid-morning and mid-afternoon are potentially associated with highest risk of onchocerciasis transmission by *S. guianense* s.l. Given the relatively low parous biting rate exhibited by this species (up to 48 flies per person-hour), it is possible that a considerable reduction of exposure to infective bites could be achieved if human-vector contact were minimized during these hours. However, this species has the highest vector competence of the three simuliids investigated, and although the distribution of the num-

ber of infective larvae per fly will be typically overdispersed (with a few flies harbouring up to 13 infective larvae), inter-individual variation in attractiveness to flies may mean that some hosts might still receive a high number of L3 larvae. The contribution of *S. incrustatum* to onchocerciasis transmission, in those localities where this species prevails, will take place mainly in the morning, whereas transmission by *S. oyapockense* s.l. in mesoendemic localities will occur throughout the day. Although this species is the least competent of the three, the hourly parous biting rate can be consistently higher than 100 per person in Maweti- and Mahekoto-theri. We have already discussed the relationship between vector competence, the vector biting rate on humans, the intensity of onchocerciasis transmission, and the endemicity of the infection in host populations in a comparative analysis between Meso-America and West Africa (Basáñez et al., 2002) and in Ecuador (Vieira et al., 2005).

4.7. Implications for ivermectin-based control

As well as describing biting patterns of parous flies and indicating times of the day most likely to contribute to onchocerciasis transmission, the information provided in this paper, once analyzed together with infection and infectivity data, will be most useful for the design of sampling protocols aimed at detecting statistically meaningful changes in the intensity of transmission during ivermectin-based control programmes. Recently, such information has been reported in the case of the Ecuadorian program for onchocerciasis control to provide clear guidelines for entomological evaluation of *S. exiguum* s.l. and *S. quadrivittatum* that will aid the Onchocerciasis Elimination Program for the Americas efforts in the region (Vieira et al., 2005). A similar analysis for the Amazonian focus will be presented elsewhere.

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