On the vectors of cutaneous leishmaniasis in the Central Amazon of Brasil. 3. Phlebotomine sand fly stratification in a terra firme forest (1)

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Abstract

Stratification studies showed that the vast majority of sand fly species in the Manaus region are found in tree canopies (15m), and that there are two dominant species which are the vectors of **Leishmania braziliensis guyanensis**. It took 43 weeks of trapping to capture all 50 species of sand flies collected.

Introduction

The systematics, ecology and behaviour of sand flies in areas where *Leishmania braziliensis guyanensis* is endemic, has been the subject of much research in the past decade. Recently the taxonomic status of the principal vector species of *"pian bois"* was clarified (Waro & Fraiha, 1977) and *Lutzomyia umbratilis* was indicated as being the principal vector both in the Monte Dourado and Manaus areas of northern Brazil (Lainson *et al.*, 1976; Arias & Freitas, 1977b). In the Manaus area, Arias & Freitas (1977a) indicated *L. anduzei* as a secondary vector of *Le*(3). *b. guyanensis* while Lainson *et al.* (1981b) indicated *L. whitmani* as a secondary vector in the Jari area. Even though the range of *L. anduzei* does extend to the Jari region, and promastigotes have been found in its digestive tract, this sand fly species has not been shown to be a secondary vector in Jari (Lainson, Shaw & Ready, personal communication). *L. whitmani*, on the other hand, has not been taken in the Central Amazon around Manaus.

Other studies of Arias *et al.* (1981), Arias & Naiff (1981) and Lainson *et al.* (1981a, 1981b) have shown that the natural reservoir host of this *Leishmania* are arboreal mammals, as suggested by Arias & Freitas (1978).

Sand fly stratification in other regions of the New World has been done by Disney (1963), Williams (1970), Chaniotis *et al.* (1971a, 1971b), Shaw *et al.* (1972), and near Manaus by Arias & Freitas (1977b). All authors showed that there were stratification differences between sand fly species, some species being caught predominantly in the canopy and other species being caught predominantly on the forest floor.

Material and methods

Modified CDC miniature light traps were utilized throughout the study. The slight modifications included replacement of the collector sack with one of a finer mesh screen and by adding a small styrofoam container containing approximately 800g. of dry ice. The styrofoam box was completely taped closed to allow slower release of the CO_2 gas, and then taped to the side of the traps. The power source for each trap was two rechargeable 6-volt motorcycle batteries. Two traps were set at 15 meters and two at 1 meter above the forest floor.

Each of the collection sites remained the same throughout the study, and were located at INPA's Ducke Forest Reserve, 26 kilometers NE of the city of Manaus on the AM-010 highway. This is a terra firme forest that is frequently entered by man and is maintained as a biological research station. A more detailed description of this area can be found in Penny & Arias (1982).

Leishmania braziliensis guyanensis has been isolated from sand flies (Arias & Freitas,

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(3) — **Le.** is used as the abbreviation for **Leishmania** to avoid confusion of **L.** for **Lutzomyia**.

1978), from wild animals (Arias *et al.*, 1981; Arias & Naiff, 1982), and man (Arias, unpublished data) from this area studied.

All sand flies were sorted at INPA in Manaus, slide mounted in Berlese solution, and identified according to the systematics proposed by Lewis *et al.* (1977) [4].

Results

Figure 1 shows the period of time that it took us to capture 100% of the flies taken in light traps. The first week we captured 46% of all species we found at the Ducke Reserve at all heights during the 62 weeks. By the second week we had taken over 50% of the species. We surpassed the 75% mark after the fifth week of captures, the 80% mark after 12 weeks, the 90% mark after 22 weeks, 95% mark after 33 weeks, and only after 43 weeks did we take all species captured during this study. No previously uncollected species were taken during the last 19 weeks of the study.

Figure 2 shows the population density of the different sand fly species taken. Even though a great variety of species were taken, the majority of individuals collected were two species. These two species are *L. umbratilis* and *L. anduzei*.

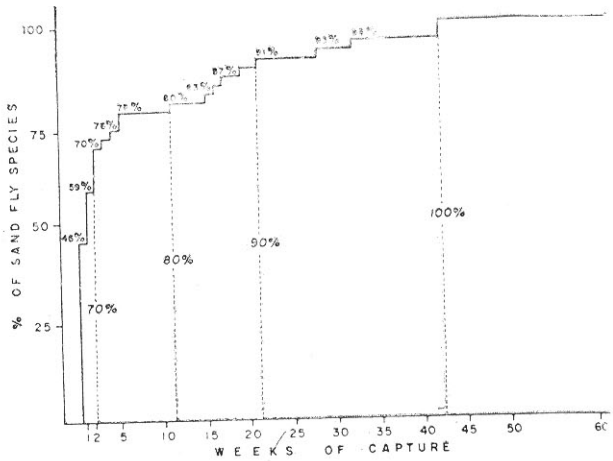

Fig. 1 — Time elapsed to capture all species of sand flies taken in light traps over a 62 week period at the Ducke Forest Reserve, Manaus, Brazil.

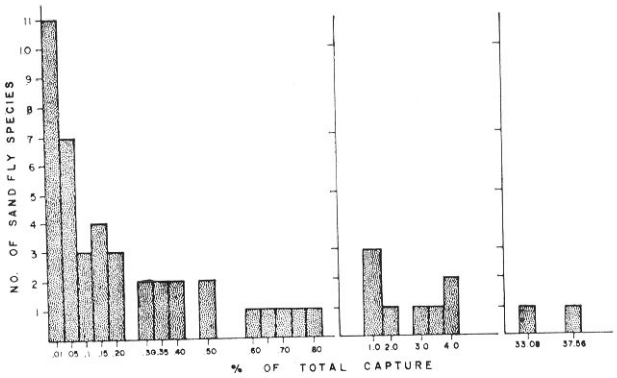

Fig. 2 — Population density of the sand fly species taken in light traps over a 62 week period at the Ducke Forest Reserve, Manaus, Brazil.

Here we can see that these two species represent over 70% of all individuals captured. Only 10 species were collected in a frequency of over 1%, and all these comprised almost 90% of the sand flies taken. The remaining 38 species comprised 10% of all sand flies trapped.

Table 1 shows the total of sand flies taken at both one and 15 meters in the study site. Only one of these, *B. pintoi*, was in the genus *Brumptomyia*, 37 species were in the genus *Lutzomyia* and 12 species were in the genus *Psychodopygus*. Three different females in the *migonei* group were taken, and it is possible that they correspond to known males of other species not taken in this study. *Shannoni* group females are those corresponding to *L. scaffi*, *L. shannoni* or *L. dendrophila*, which at present we cannot separate. Five species are cited as "*like*" or with a number and are being studied further and will be described separately. The total number of sand flies collected was 21,026, of which 18,215 (86.6%) were taken at 15 meters, and only 2,811 (13.4%) were taken at one meter. Only five species were taken at the one meter height, and not at the 15 meter level. These are: *P. chagasi*, of which only one male was taken at one meter; *L. evangelistai*, also of which only one male specimen was taken at one meter; *L. flaviscutelata* with five males taken at one

(4) — Since the initial writing of this manuscript the authors accept **Psychodopygus** as a valid genus, as included here.

TABLE 1 — Species composition and frequency of sand flies taken over a 62 week period at the Ducke Forest Reserve, Manaus, Brazil.

	1 Mt	%	15 Mts	%	Total	%
Brumptomyia pintoi	1	.04	2	.01	3	.01
Lutzomyia abonnenci	0	0	20	.11	20	.10
L. anduzei	418	14.87	7479	41.06	7897	37.56
L. antunesi	0	0	1	.01	1	<.01
L. aragaoi	23	.82	153	.84	176	.84
L. bacula	0	0	1	.01	1	<.01
L. begonae	71	2.53	27	.15	98	.47
L. campbelli	0	0	2	.01	2	<.01
L. dasymera	0	0	1	.01	1	<.01
L. dendrophyla	0	0	6	.03	6	.03
L. dreisbachi	0	0	3	.02	3	.01
L. evangelistai	1	.04	0	0	1	<.01
L. flaviscutellata	5	.18	0	0	5	.02
L. furcata	15	.53	52	.29	67	.32
L. inpai	1	.03	0	0	1	<.01
L. longispina (like; n. sp.)	7	.25	23	.13	30	.14
L. lutziana	0	0	3	.02	3	.01
L. monstruosa	58	2.06	5	.03	63	.30
L. nordestina (like)	49	1.74	13	.07	62	.29
L. olmeca nociva	96	3.42	6	.03	102	.49
L. pacae	2	.07	0	0	2	<.01
L. pilosa	4	.14	6	.03	10	.05
L. punctigeniculata	0	0	1	.01	1	<.01
L. rorotaensis	351	12.49	416	2.28	767	3.65
L. ruii	239	8.50	575	3.16	814	3.87
L. runoides	0	0	1	.01	1	<.01
L. sericea	31	1.10	42	.23	73	.35
L. shannoni	7	.25	27	.15	34	.16
L. spathotricha	6	.21	70	.38	76	.36
L. spinosa	0	0	6	.03	6	.29
L. triacantha	4	.14	4	.02	8	.04
L. trichopyga	18	.64	118	.65	136	.65
L. trispinosa	4	.14	15	.08	19	.09
L. tuberculata	49	1.74	102	.56	151	.72
L. umbratilis	395	14.05	6560	36.01	6955	33.08
L. williamsi	0	0	24	.13	24	.11
L. 1.01.2.06	31	1.10	4	.02	35	.17
L. 1.01.2.09	0	0	1	.01	1	<.01
Série Cruciata ♀♀	51	1.81	102	.56	153	.73
Gp. Migonei ♀♀	4	.14	2	.01	6	.03
Gp. Migonei ♀♀	0	0	4	.02	4	.02
Gp. Migonei ♀♀	4	.14	3	.02	7	.03
Sg. Pressatia ♀♀	29	1.03	83	.46	112	.53
Gp. Shannoni ♀♀	7	.25	45	.25	52	.25
L. sp ♂♂ ♀♀	140	4.98	174	.96	314	1.49
Psychodopygus amazonensis	8	.28	31	.17	39	.19
P. ayrozai	24	.85	279	1.53	303	1.44
P. bispinosa	2	.07	8	.04	10	.05
P. chagasi	1	.04	0	0	1	<.01
P. claustrei	68	2.42	92	.51	160	1.31
P. corossoniensis	11	.39	154	.85	165	.78
P. davisi	49	1.74	598	2.19	647	3.08

TABLE 1 — (Continuação).

	1 Mt	%	15 Mts	%	Total	%
P. guyanensis	14	.50	219	1.20	233	1.11
P. hirsutus	1	.04	28	.15	29	0.14
P. paraensis	27	.96	306	1.68	333	1.58
P. squamiventris squamiventris	407	14.48	178	.98	585	2.78
P. n. sp. near **davisi**	0	0	5	.03	5	.02
P. sp. ♀♀	78	2.77	135	.74	213	1.01
	2.811		18.215		21.026	

meter; *L. inpai,* also with only one male taken at one meter; and *L. pacae,* of which two individuals were taken at one meter. On the other hand, 11 species were taken at 15 meters, yet not at one meter. These taken at 15 meters were: *L. abonnenci* (20), *L. antunesi* (1), *L. bacula* (1), *L. campbelli* (2), *L. dasymera* (1), *L. dendrophila* (6), *L. dreisbachi* (3), *L. lutziana* (3), *L. punctigeniculata* (1), *L. runoides* (1). *L. spinosa* (6), *L. williamsi* (24), species 1.01.2.09 (1), (in the subgenus *Lutzomyia),* and *P.* n. sp. near *davisi* (5).

At the one meter height, there were 5 dominant species which comprised almost 65% of the total sand fly catches. These were: *L. anduzei,* which comprised 14.82%, *L. rorotaensis,* 12.49%, *L. ruii,* 8.50%, *L. umbratilis* 14.50% *P. s. squamiventris* 14.48%.

On the other hand, there were only two species which could be considered dominant at the 15 meter capture sites. These were: *L. anduzei,* which comprised 41.06% and *L. umbratilis,* which comprised 36.01%.

Of the total sand fly population captured at both heights, we can see that there are two dominants, *L. anduzei* and *L. umbratilis,* having captured 37.56% and 33.08% respectively.

Figure 3 shows the incidence of all sand flies per trap per week as caught at the one and 15 meter heights. We see here that there appears to be a high initial population which drops readily after 5 weeks of trapping and has peaks during the end of the month of November and month of December, 1977 and a larger peak during the month of May, 1978

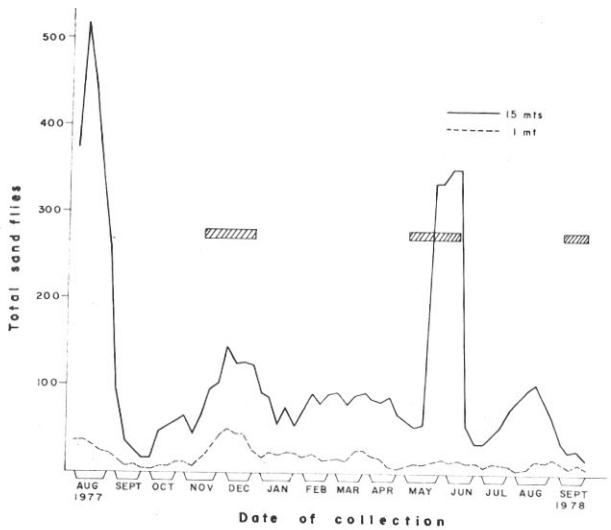

Fig. 3 — Seasonal distribution of all sand flies captured in light traps over a 62 week period at the Ducke Forest Reserve, Manaus, Brazil (from Penny & Arias, 1982). The curves represent 4 week moving averages as in Chaniots **et al.** (1971b).

However, comparing this Figure with Figure 4 *(L. anduzei)* and Figure 12 *(L. umbratilis),* we see that the peak influences are due to the high number of specimens of these two species. During the month of May, 1978 there is a large number of individuals of these two species in one 15 meter trap during a two week period. One of these trap nights had over 4000 sand flies of which most were of one of these two species. The population of all sand flies captured at the one meter traps showed no significant fluctuation except for a slight peak during the month of December, 1977. The

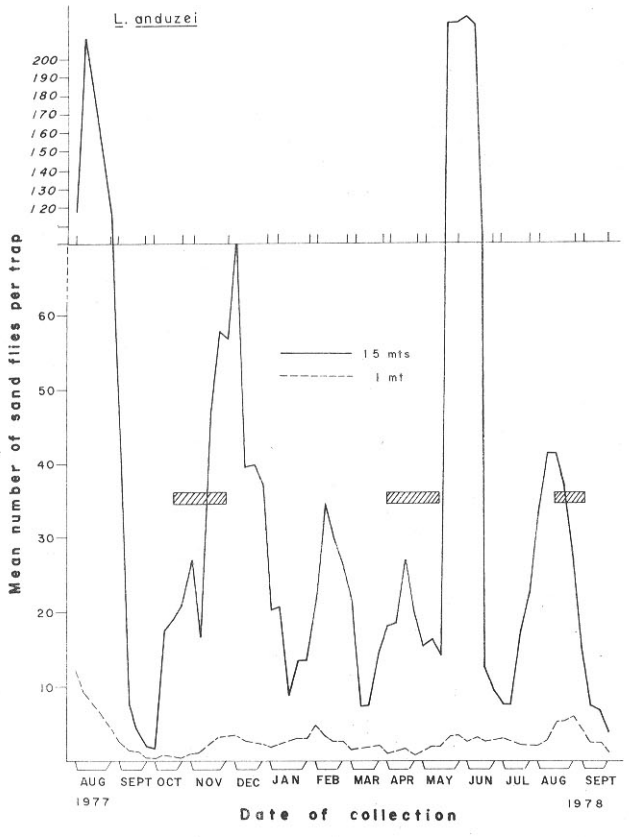

Fig 4 — Seasonal distribution of **L. anduzei** captured in light traps over a 62 week period at the Ducke Forest Reserve, Manaus, Brazil. The curves represent 4 week moving averages as in Chaniotis **et al.** (1971b).

solid bars across the graphs at three different intervals represent periods of time when twice as many traps (4) were set at both heights to assess "trapping out", which did not appear to occur. The peak of sand fly activity at both heights during the end of November and throughout the month of December may be due to the second complement of traps set. This may account for a large existing adult population (particularly *L. anduzei* and *L. umbratilis)* which is taken initially, and subsequent captures represent the forest productivity of sand flies. One second possibility for an increase in population at this time is the change in season, beginning of rainy season, which has been shown to trigger activity in some insect families (Penny & Arias, 1982).

Figure 4 shows the seasonal incidence of *L. anduzei*. It must be noted here that the graph is shown with two scales on the left hand side to reduce the size of the graph and maintain the smaller peaks. It can be seen here that the initial population appears to drop rapidly after an initial peak, slowly rising around November of 1977, dropping at the end of December, and having another large peak in May of 1978. The one meter population appears to be at its highest during the first part of the collection period, dropping down and maintaining a low level of incidence throughout the collection time.

Figure 5 shows the activity of *P. ayrozai*. This species had two peaks of activity around December, 1977 and March, 1978 at the 15 meter height. At the one meter height this species was not present at all times and had a slight peak of activity during March, 1978.

Figure 6 shows the population activity of *P. davisi*. This species has a main peak of activity at 15 meters during the month of February and lesser peaks of activity throughout the year. At one meter the activity of this species appears to be slight throughout the capturing period.

Figure 7 shows the activity of *P. guyanensis*. At both the 15 and the one meter heights this species was nonapparant until November, 1977. This activity is noted until around August of 1978 when it stops. At one

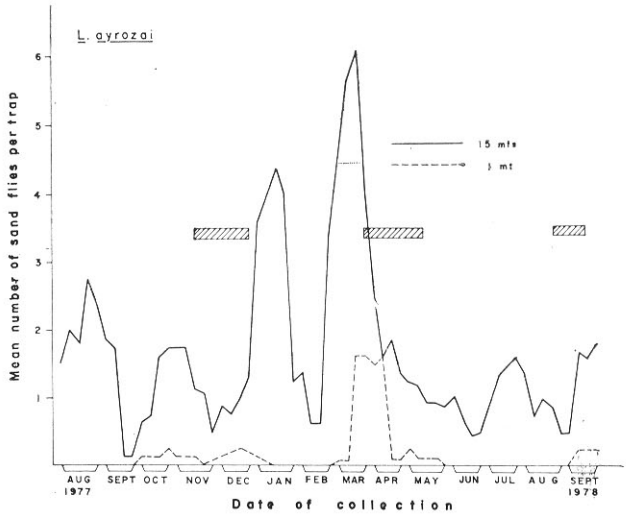

Fig. 5 — Seasonal distribution of **P. ayrozai** captured in light traps over a 62 week period at the Ducke Forest Reserve, Manaus, Brazil. The curves represent 4 week moving averages as in Chaniotis **et al.** (1971b).

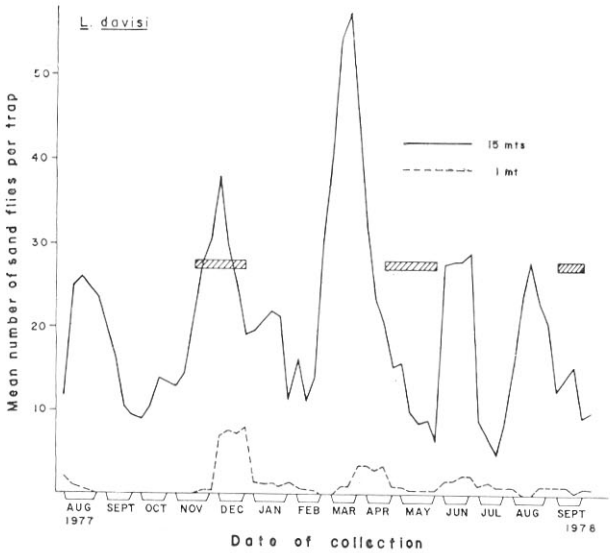

Fig. 6 — Seasonal distribution of **P. davisi** captured in light traps over a 62 week period at the Ducke Forest Reserve, Manaus, Brazil. The curves represent 4 week moving averages as in Chaniotis **et al.** (1971b).

meter this species appears in low number and follows approximately the same activity but at greatly reduced levels; it was not captured again after July, 1978. The low periods of activity of *P. guyanensis* correspond directly with the dry season in our area (Penny & Arias, 1982).

Figure 8 shows the population activity of *P. paraensis*. Again at the 15 meter level the peak activity is during the month of February. This species has two subsequent peaks of activity during the latter part of the year. The initial population, like that of *L. anduzei* and *L. umbratilis*, started high only to drop rapidly. At one meter height the population activity was low, sporadically appearing in the captures.

Figure 9 shows the population activity of *L. rorotaensis*. This species was one of the few species which was considerably more active at the one meter level than at the 15 meter level. At the 15 meter level this species was present at a low incidence, having a slight peak of activity during the end of 1977. At the one meter height traps it was collected in great numbers and showed a small peak of activity during October, 1977. It had its greatest peak of activity during November-December, 1977. After this, the population dropped only to have a slight peak of activity during late June of 1978.

Figure 10 shows the activity of a new species of sand fly in the subgenus *Trichophoromyia* (Arias & Young, 1982), which for now is called 1.20.1.20. As *L. anduzei*, *P. paraensis* and *L. umbratilis*, this species shows great activity at the beginning of the trapping program and drops after the first 5 weeks. The two following peaks of activity at the 15 meter level occur during the months of November, 1977 and March, 1978. This is one of the few species which showed considerable activity at both the one and 15 meter levels, even though there was greater activity at the 15 meter level. Most of the one meter level activity peaks correspond with those at the 15 meter level.

Figure 11 shows the activity of *P. s. squamiventris*. The 15 meter activity was low and fairly constant throughout the capture program. However, at the one meter level the activity was more pronounced. Here we find activity peaks during late November — early December, 1977 and late March and early April, 1978. This species, once considered to be *P. maripaensis* by Arias & Freitas (1977a, 1977b, 1978), has recently been shown to be *P. s. squamiventris* by Ready *et al.* (1982).

Figure 12 shows the activity of *L. umbratilis*. As mentioned before, this species showed

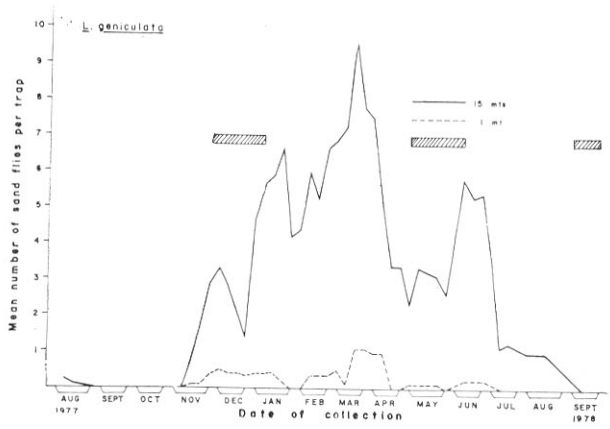

Fig. 7 — Seasonal distribution of **P. guyanensis** captured in light traps over a 62 week period at the Ducke Forest Reserve, Manaus, Brazil. The curves represent 4 week moving averages as in Chaniotis **et al.** (1971b).

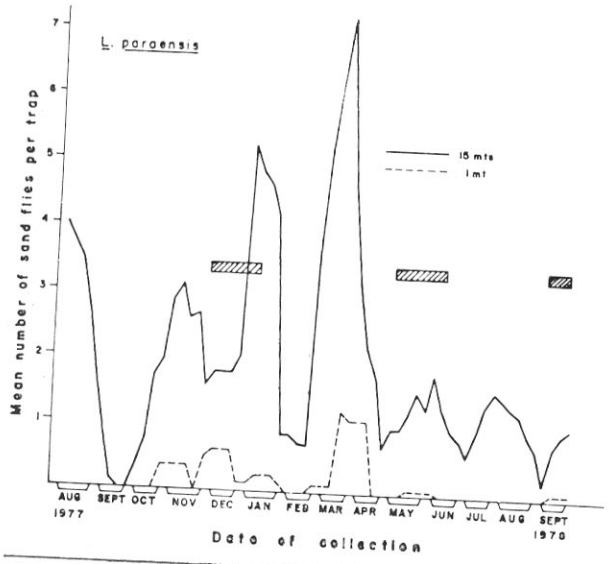

Fig. 8 — Seasonal distribution of **P. paraensis** captured in light traps over a 62 week period at the Ducke Forest Reserve, Manaus, Brazil. The curves represent 4 week moving averages as in Chaniotis **et al.** (1971b).

high population activity at the beginning of the trapping program, a lesser peak of activity in late November — early December, 1977 and a high peak of activity during May, 1978 where the catches in two trap nights were extremely high. At the one meter level the population activity appears to be constant and without much significant change.

The seasonal populational activity for the remaining species was so low that only sporadic captures were found and no conclusions could be drawn.

Discussion

At least 22 weeks of trapping were required to get 90% of the species of sand flies utilizing CO_2 baited CDC light traps. Even though we ran our traps for 62 weeks, and we had captured all the species by the 43rd week, we did not capture all the species found in the area. Other studies in the same Ducke Reserve area have produced at least 5 other species which we did not capture here, and we expect that in the future we will find more species as new capturing techniques are applied; collections of some sand fly species being very specific as to capture method. *L. dendrophila* is a species that is very commonly taken in tree base captures, yet we only took 6 individuals in light traps and these were taken at 15 meters. *L. spinosa*, another species found resting on tree bases. was also only taken in traps at 15 meters.

The relatively small number of commonly captured sand flies, and the large number of infrequently taken species reflects what appears to be the trend of species compositions in the Amazonian forest (New, 1979; Meinander & Penny, 1982). Very few species are taken very commonly and many species are hard to find. The capture method utilized also strongly influences the species taken. However, summarizing all methods of capture we have utilized in the past (Arias & Freitas. 1977a, 1977b, 1978), there still exists a vast number of species that are not at all frequent.

Since the two dominant species of sand flies captured during this program were the vectors of "pian bois" in the Manaus region of the Amazon (Arias & Freitas, 1977a, 1978), the predominance of these species at the canopy level (15 meters) of the terra firme forest, is of great significance considering the recent findings in the identifications of the natural reservoir hosts of *Leishmania braziliensis guyanensis*. *It* has been shown that the sloth is the principal silvatic natural reservoir host (Lainson *et al.*, 1981a) and that the opossum is a principal natural reservoir host in disturbed terra firme forest (Arias & Naiff

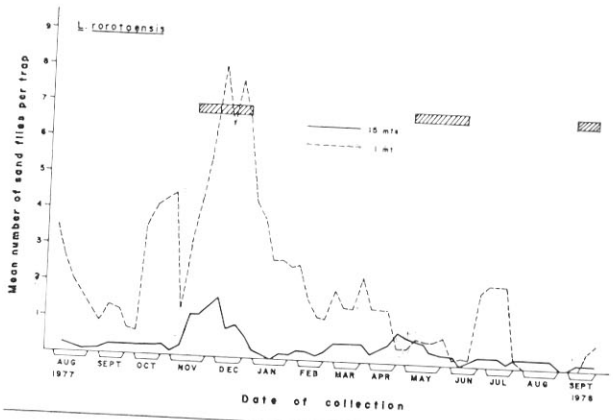

Fig. 9 — Seasonal distribution of **L. rorotaensis** captured in light traps over a 62 week period at the Ducke Forest Reserve, Manaus, Brazil. The curves represent 4 week moving averages as in Chaniotis **et al.** (1971b).

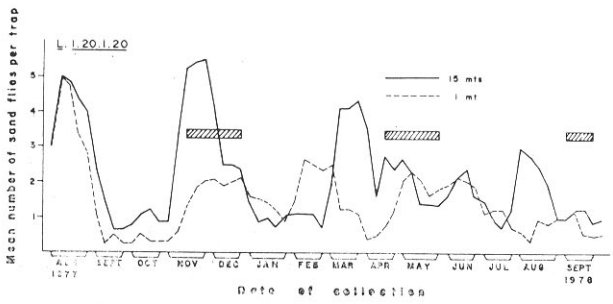

Fig. 10 — Seasonal distribution of **L. 1.20.1.20** captured in light traps over a 62 week period at the Ducke Forest Reserve, Manaus, Brazil. The curves represent 4 week moving averages as in Chaniotis **et al.** (1971b).

1982). Human bait captures in terra firme forest near Manaus also show that even though *L. umbratilis* is a causual man biter at the forest floor, it is the principal man biter at the 15 meter level (Arias & Freitas, 1977b; Ready & Arias, unpublished data). Vector and anthropophilic species have been shown to have different biting and population peaks at different heights in the forest from Belize, British Honduras (Disney, 1968; Williams, 1970), and Panama (Johnson *et al.*, 1963; Thatcher, 1968; Chaniotis *et al.*, 1971a, 1971b) to Brazil (Shaw *et al.*, 1968; Arias & Freitas 1978). Usually it has been noted that the biting habits of the vector species is very closely related to the habitats of the natural reservoir host. If the natural reservoir host is a ground dweller, the vector species usually is a ground level biter; and if the natural reservoir host is a tree climber, the vector species is a canopy biter (Shaw *et al.*, 1968; Arias & Freitas, 1978).

Even though there is a stratificational preference of sand flies, it appears from these results that the sand flies in the Northern Central Amazon prefer the canopy to the forest floor. Of all the species, only five were more frequently caught on the one meter traps. This appears to be in contrast with the results of light trapping in Panama (Chaniotis *et al.*, 1971b) where only two species were more frequently captured in the canopy light traps. In our work, almost 90% of all sand flies taken were in the canopy light traps, while in the Panama work less than 25% were taken in canopy light traps. Maybe the influence of CO_2, which was present in our traps and not in theirs, accounts for these differences. In another work (Chaniotis *et al.* 1971a), they showed that with man biting captures almost 50% were taken at the canopy level.

Several times our study showed that there was an initial population peak that sharply fell during the first five weeks. Subsequently there was a smaller peak during the month of October. It is believed that the original population is high and the light traps rapidly reduce this, in the immediate area and the subsequent collections are indicators of lateral migration through the forest and emergence of new sand flies. We feel this is the case because the smaller peak in October corresponds to the introduction of the 4 additional traps that were set out to see if we were "trapping out" the population. The first few catches in the extra sets of traps were usually higher than the traps that remained throughout the entire study.

The large catches which correspond to the very large peak in May, 1978 may have resulted from a "bonanza" population (Penny & Arias, 1982). There was only one trap at 15 meters which had this exceptionally high number of sand flies, particularly *L. anduzei* and *L. umbratilis;* this was during two consecutive weeks, the first week with over 4000 sand flies in the trap. *P. s. squamiventris* is the most avid man biter at ground level, yet does not appear

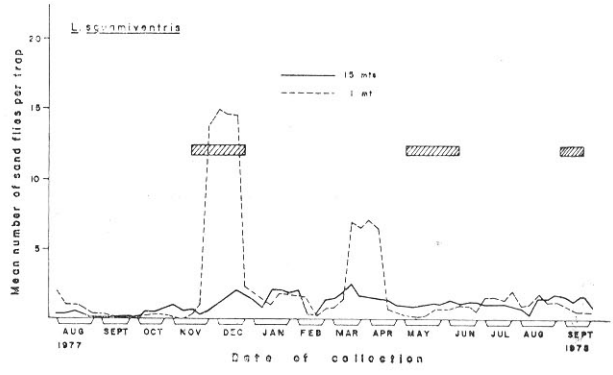

Fig. 11 — Seasonal distribution of **P. s. squamiventris** captured in light traps over a 62 week period at the Ducke Forest Reserve, Manaus, Brazil. The curves represent 4 week moving averages as in Chaniotis **et al.** (1971b).

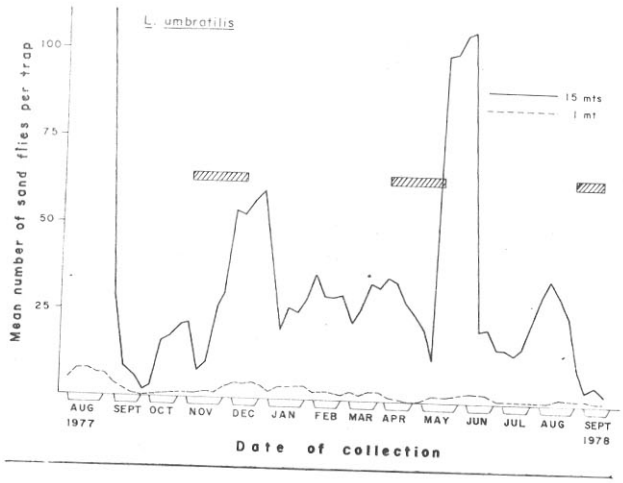

Fig. 12 — Seasonal distribution of **L. umbratilis** captured in light traps over a 62 week period at the Ducke Forest Reserve, Manaus, Brazil. The curves represent 4 week moving averages as in Chaniotis **et al.** (1971b).

to go up into the canopy except in relatively small numbers. The peak activity in the traps corresponds with those of Arias & Freitas (1977b) when doing horse and human bait studies.

The most frequent peak of specific activity is that in late November and throughout December. This may be due to one of two factors. First, that the second complement of traps encountered an older population that had not been previously taken. If this were true, it is logical that the peak would not be as accentuated as the initial peak because a) there is a dilution factor of 2 other traps (those which had been running for a longer period of time) and b) because of the use of a 4 week running mean, which tends to reduce extreme "peaks and valleys". The second possible explanation for this peak is that there is a slight emergence of insects in general at this time of year (Penny & Arias, 1982) which closely corresponds with the onset of the rainy season.

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Resumo

Estudos de estratificação mostram que a maioria das espécies de flebótomos, na região de Manaus é encontrada na copa da floresta, espécies das quais duas são dominantes, funcionando como vetores de **Leishmania braziliensis guyanensis**. Gastaram-se 43 semanas de capturas para coletar todas as 50 espécies encontradas e é de supor-se existir uma população de flebótomos por espécie na floresta.

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