LEISHMANIASIS IN BRAZIL: VI. OBSERVATIONS ON THE SEASONAL VARIATIONS OF LUTZOMYIA FLAVISCUTELLATA IN DIFFERENT TYPES OF FOREST AND ITS RELATIONSHIP TO ENZOOTIC RODENT LEISHMANIASIS (LEISHMANIA MEXICANA AMAZONENSIS).

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Introduction

The fauna of a particular area of forest is influenced by a number of natural variables ranging from rainfall to the relative abundance of food. In such a forest the existence of a focus of enzootic rodent leishmaniasis depends upon a degree of stability of the rodent and sandfly population throughout the year and there must be a continual supply of sandflies and non-immune rodents during this period to maintain the enzootic. Clearly, then, it is important to study the seasonal variations of suspected vectors, since a marked fall in their numbers at a certain time of year might reduce the possibility of transmission.

In Central America it would appear that the phlebotomine population in general is higher during the wet season and tends to decline during the dry season (STRANGEWAYS-DIXON and LAINSON, 1966; THATCHER and HERTIG, 1966; CHANIOTIS et al., 1971). Such species as Lu. trinidadensis, Lu. shannoni, Lu. ylephilatrix and Lu. carpenteri (FAIRCILD and HERTIG, 1948, 1950, 1952, 1953) appear to be common throughout the year. Others such as Psychodopygus panamensis and Lu. olmeca appear to be primarily wet season species (FAIRCILD and HERTIG, 1951; BIAGI and BIAGI, 1953; DISNEY, 1968); Lu. permira, on the other hand would seem to be an essentially dry season species. DISNEY (1968) trapped Lu. cruciata fairly regularly throughout the year but found that this species tended to peak towards the middle of the dry season.

To date there have been few observations on seasonal fluctuations of sandflies in forested regions of South America. GUIMARAES et al. (1968) studied the sandflies caught in animal burrows and tree trunks in the Utinga forest but the presentation of the results makes it difficult to assess any seasonal variations, especially as they do not include the months of the year with the highest rainfall.

It would appear that seasonal variations have been basically studied in two different ways. Some authors have considered individual species while others have considered the sandfly population as a whole. In studying the epidemiology of leishmaniasis it would seem more profitable to analyse the seasonal fluctuations of species which are considered to be vectors rather than a variety of species collected by non-specific methods such as light traps and non-baited suction devices.

The purpose of the present paper is to report the observations on the seasonal variations of sandflies attracted to rodents in two different types of forest and to relate this to the incidence of the appearance of infections of Leishmania mexicana amazonensis in Oryzomys capito.
Materials and methods

a) Seasonal variations of sandflies.

We have previously noted (SHAW and LAINSON, 1968) that the distribution of sandflies in the forest is very uneven, some traps consistently catching more flies than others. If, under these conditions, there is a marked fall in the sandfly population then the chances of detecting flies will be greater in the traps set in the higher density areas. To avoid the problem of erroneously concluding that sandflies do not occur in certain months, by placing traps in low density areas, we ran a small trapping programme to determine what sites gave higher catches. Our traps for the long term studies were then set in locations that continuously yielded more sandflies.

Rodent-baited oiled traps, as described by DISNEY (1966), were baited with spiny rats (Proechimys guyannensis) and hung so that the oiled tray was approximately 20 cm. above the ground. The number of males and females of each species caught each night were recorded and monthly means for each species were calculated by dividing the total caught each month by the number of nights on which traps had been set. On occasional nights it was noted that there were exceptionally high catches; HADDOW (1960) suggested the use of William's modification of the Geometric Mean \((GM^w)\) in dealing with such extreme values. We, therefore, calculated the GM\(^w\) for each month as well as the arithmetic mean.

\[
\log (n + 1) \quad \text{where } n_1, n_2, \ldots \text{ are the actual values of a series of } N \text{ observations.}
\]

Traps were set in the Utinga forest at location R.8d (SHAW and LAINSON, 1968) and in the Catu forest at location 2 (Table II). The Utinga area was described by us in 1968 (LAINSON and SHAW, 1968) and all we might add to this is that trap R.8d was located on an edge of “terra firme” forest which is seasonally flooded. Catu is a typical area of “igapo” forest which is completely flooded for most of the year. It is in the grounds of the local Agricultural Research Station, Instituto de Pesquisas Agropecuárias do Norte (I.P.E.A.N.). Location 2 in this forest was at the base of a large tree that had entangled stilt roots. A few traps were set for shorter periods in the Area de Pesquisas Ecológicas do Guamá (A.P.E.G.), which is a forest similar in character to Utinga; in the Aurá forest of the Agricultural station, which is an area of high “terra firme” forest; and in another area of “igapo” forest near the Água Preta reservoir.

The seasonal fluctuations of \(Lu. flaviscutellata\) in Utinga were plotted in graphical form (Fig. 1) against the total monthly rainfall. The rainfall figures used were those of the Utinga field station which is about 1 km. from the study area. The average monthly rainfall for a period of three years is also given and the average nightly catches for each month in the Catu forest are plotted against the total monthly rainfall (Fig. 2). In this case, however, we used the rainfall figures for the A.P.E.G. reserve, which is about 2 km. from the Catu reserve. A 10 year average monthly rainfall for I.P.E.A.N. is also plotted on the Catu figures.

b) Infection rates in rodents.

We had difficulty in choosing a method of assessing firstly the infection rate and secondly the monthly rodent population. Dr. J. P. Woodall, Director of the Belém Virus Laboratory, kindly made available to us animals that were caught in a mammal re-trapping programme in the Catu and A.P.E.G. forests. Any lesions were examined for the presence of leishmania and the animal was recorded as being positive only in the month when it was diagnosed. If recaptured in subsequent months it was not counted as being positive even if the lesions still had parasites.

The numbers of positive animals were then recorded as percentages of the total number caught each month. For this study we limited our observations to one species of rodent, Oryzomys capito, as there is a considerable amount of data available on the biology of this particular species. In this way we arrived at a figure which represented the number of newly infected animals per month.

c) Dissections of sandflies other than \(Lu. flaviscutellata\).

Up to now the only species of sandfly that we had examined in detail for infection with \(L. m. amazonensis\) was \(Lu. flaviscutellata\), and to determine the possible importance of other sandflies as vectors we now dissected the females of the species caught in relatively small numbers (\(Lu. infraspinosa, Ps. rooti, Lu. saudensis, Lu. monstrosa,\) and \(Lu. antunesi\)).
Results

a) The seasonal fluctuations of sandflies.

The numbers of *Lu. flaviscutellata* caught in Catu and Utinga are given in tables I and II. From these figures it can be seen that the catches of *Lu. flaviscutellata* were greater in Catu than in Utinga, there being an average of 79.4 flies per night in Catu and 23.7 in Utinga over the respective study periods.

Similarly, more *Lu. flaviscutellata* were captured in the wetter Agua Preta forest than in the drier forests of Aurá and A.P.E.G. *Lu. infraspinosa*, *Ps. rooti*, *Lu. antunesi*, *Lu. saulensis* and *Lu. monsturosa* were taken in all the sites but more *Lu. saulensis* and *Lu. monsturosa* were caught in the wetter Catu forest. *Lu. gomezi*, *Ps. paraensis* and *Lu. pacae* were only captured in the drier forests (Utinga, Aurá, and A.P.E.G.).

![Graph](image-url)

**Fig. 1.** The relationship of the average nightly catches of *Lu. flaviscutellata*, caught in the Utinga forest, to rainfall.

From figs. 1 and 2 it can be seen that the seasonal fluctuations in both Catu and Utinga follow a similar pattern in relation to rainfall. The rains begin to increase during November and December, reaching a peak around March and April: correspondingly, *Lu. flaviscutellata* catches begin to fall off with the onset of the rainy season and only begin to increase again when the excessively wet period stops. During the drier season, from June to November, the *Lu. flaviscutellata* population increases and appears to reach a peak around December. It seems that the critical rainfall level in Catu is around 200
to 250 mm. per month, for when the rainfall is greater than this the numbers caught begin to decline. In the Utinga area, it would appear that it is also around 200 to 250 mm. per month, as the excessively high rainfall in November 1968 also led to a drop in the *Lu. flaviscutellata* population during this period.

![Graph](image)

**Fig. 2.** The relationship of the average nightly catches of *Lu. flaviscutellata*, caught in the Catu forest, to rainfall.

Certain traps caught more rare species than others. In Utinga, for example, trap 9 caught more *Lu. infraspinosa* than *Lu. flaviscutellata*, but the proportion of males to females was almost equal, indicating the trap was probably very close to a breeding site.

In Catu the following species were caught in small numbers, representing approximately 2% of the total catch (the number of each species caught per 10 trap nights is given and the figure in brackets represents the proportion of males to females:—*Lu. infraspinosa* 14.3 (1 : 1.5); *Ps. rooti* 3.2 (1 : 9.59); *Lu. saulensis* 1.5 (1 : 8.44); *Lu. monsturosa* 1.2 (1 : 2.14); *Lu. antunesi* 1.2 (1 : 0.68); *Lu. pinottii* 0.2 (1 : 0.71). In general it was difficult to determine any seasonal trends for the above mentioned species in view...
of the small numbers caught each month, *Lu. infraspinosa*, *Lu. antunesi* and *Lu. montistuosa*, however, were captured in greater numbers towards the end of the dry season (September to January), while *Ps. rooti* showed no particular seasonal fluctuations. Such species as *Lu. furcata*, *Lu. pinotti*, *Lu. paca* and *Lu. tricentra* were caught in such small numbers that it is not worth quoting figures and it is impossible to detect any seasonal trends.

Table I. Catches of *Lutzomyia flaviscutellata* caught in Disney traps set at location 8 in the Utinga forest, Belém, Pará, between August 1967 and December 1968.

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<tr>
<th>Month</th>
<th>Aug</th>
<th>Sep</th>
<th>Oct</th>
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<th>May</th>
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<th>Jul</th>
<th>Aug</th>
<th>Sep</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. trap nights</td>
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<td>12</td>
<td>8</td>
<td>17</td>
<td>15</td>
<td>18</td>
<td>15</td>
<td>14</td>
<td>17</td>
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<td>14</td>
<td>18</td>
<td>17</td>
<td>17</td>
<td>18</td>
<td>17</td>
<td>16</td>
<td>259</td>
</tr>
<tr>
<td>Total catch</td>
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<td>281</td>
<td>233</td>
<td>687</td>
<td>244</td>
<td>887</td>
<td>255</td>
<td>494</td>
<td>164</td>
<td>107</td>
<td>183</td>
<td>278</td>
<td>194</td>
<td>531</td>
<td>444</td>
<td>441</td>
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<tr>
<td>Arithmetic mean</td>
<td>9.5</td>
<td>23.4</td>
<td>29.1</td>
<td>40.4</td>
<td>16.3</td>
<td>49.3</td>
<td>17.0</td>
<td>35.3</td>
<td>9.6</td>
<td>5.9</td>
<td>13.1</td>
<td>15.4</td>
<td>11.4</td>
<td>31.2</td>
<td>24.7</td>
<td>25.9</td>
<td>40.2</td>
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<tr>
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<td>38.7</td>
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<td>38.7</td>
<td>15.0</td>
<td>28.1</td>
<td>7.9</td>
<td>5.1</td>
<td>12.2</td>
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<td>10.6</td>
<td>29.8</td>
<td>22.9</td>
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<td>33.08</td>
<td>10.61</td>
<td>23.50</td>
<td>6.02</td>
<td>3.53</td>
<td>9.05</td>
<td>8.43</td>
<td>6.12</td>
<td>11.65</td>
<td>19.39</td>
<td>15.07</td>
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<tr>
<td>Total Geometric mean (Mw)</td>
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<td>27</td>
<td>15</td>
<td>29</td>
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<td>191</td>
<td>30</td>
<td>101</td>
<td>30</td>
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<td>12</td>
<td>3</td>
<td>13</td>
<td>25</td>
<td>32</td>
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<td>1.7</td>
<td>2.5</td>
<td>10.6</td>
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<td>7.2</td>
<td>1.8</td>
<td>0.9</td>
<td>0.9</td>
<td>0.2</td>
<td>0.8</td>
<td>1.5</td>
<td>1.8</td>
<td>5.2</td>
<td>6.9</td>
<td>48.6</td>
</tr>
<tr>
<td>Geometric mean (Mw)</td>
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<td>2.11</td>
<td>1.27</td>
<td>1.18</td>
<td>1.36</td>
<td>8.48</td>
<td>1.15</td>
<td>4.23</td>
<td>1.19</td>
<td>0.48</td>
<td>0.49</td>
<td>0.01</td>
<td>0.53</td>
<td>0.99</td>
<td>1.17</td>
<td>4.07</td>
<td>5.07</td>
<td>34.07</td>
</tr>
<tr>
<td>% of total monthly catches</td>
<td>3.9</td>
<td>1.6</td>
<td>6.4</td>
<td>4.2</td>
<td>15.2</td>
<td>21.5</td>
<td>11.8</td>
<td>20.4</td>
<td>18.3</td>
<td>15.0</td>
<td>6.6</td>
<td>1.08</td>
<td>6.7</td>
<td>4.7</td>
<td>7.2</td>
<td>20.0</td>
<td>17.1</td>
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</tr>
</tbody>
</table>

Table II. Catches of *Lutzomyia flaviscutellata* caught in Disney traps set at location 2 in the Catu forest, Belém, Pará, between October 1968 and November 1969.

<table>
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<th>Month</th>
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<th>Dec</th>
<th>Jan</th>
<th>Feb</th>
<th>Mar</th>
<th>Apr</th>
<th>May</th>
<th>Jun</th>
<th>Jul</th>
<th>Aug</th>
<th>Sep</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. trap nights</td>
<td>36</td>
<td>33</td>
<td>32</td>
<td>36</td>
<td>31</td>
<td>32</td>
<td>40</td>
<td>35</td>
<td>30</td>
<td>36</td>
<td>32</td>
<td>36</td>
<td>36</td>
<td>32</td>
<td>477</td>
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</tr>
<tr>
<td>Total catch</td>
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<td>2,377</td>
<td>4,300</td>
<td>3,274</td>
<td>2,358</td>
<td>1,138</td>
<td>1,358</td>
<td>1,241</td>
<td>1,638</td>
<td>2,169</td>
<td>2,559</td>
<td>3,251</td>
<td>3,916</td>
<td>3,565</td>
<td>37,855</td>
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<tr>
<td>Arithmetic mean</td>
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<td>72.03</td>
<td>134.37</td>
<td>90.94</td>
<td>76.06</td>
<td>35.56</td>
<td>33.95</td>
<td>35.46</td>
<td>54.6</td>
<td>60.25</td>
<td>79.97</td>
<td>90.31</td>
<td>108.78</td>
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<td>3,003</td>
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<td>1,853</td>
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<td>898</td>
<td>1,160</td>
<td>1,474</td>
<td>1,852</td>
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<td>3,304</td>
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<td>21.51</td>
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<td>51.84</td>
<td>37.51</td>
<td>68.29</td>
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<td>980</td>
<td>505</td>
<td>243</td>
<td>334</td>
<td>343</td>
<td>478</td>
<td>695</td>
<td>707</td>
<td>943</td>
<td>612</td>
<td>600</td>
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</tr>
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<td>Arithmetic mean</td>
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<td>16.91</td>
<td>40.53</td>
<td>27.22</td>
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<td>7.59</td>
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<td>5.74</td>
<td>13.29</td>
<td>13.64</td>
<td>17.46</td>
<td>20.66</td>
<td>13.98</td>
<td>16.72</td>
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<tr>
<td>% of total monthly catches</td>
<td>28.9</td>
<td>23.5</td>
<td>30.2</td>
<td>29.9</td>
<td>21.4</td>
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<td>27.4</td>
<td>29.0</td>
<td>15.6</td>
<td>16.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

b) *Seasonal incidence of leishmaniasis in Oryzomys capito*

The number of new infections diagnosed each month in *O. capito* is expressed as a percentage of the monthly catch (Fig. 3). There was a fall in the number of new infections during the middle of the year, around June and July. The number of *Oryzomys* caught each month showed no particular pattern in relation to rainfall, in fact the lowest infection rates were observed during the months of May, June and July 1969, when some of the highest catches were made. There was no relationship between the numbers caught and the incidence of initial infections.
c) Dissections of sandflies other than Lu. flaviscutellata

A total of 731 females was dissected: 461 Lu. infraspinosa, 126 Ps. rooti, 66 Lu. saulensis, 55 Lu. monsturosa and 37 Lu. antunesi. Various flagellate infections, which will be discussed in greater detail in a future publication, were seen in 6 Lu. infraspinosa, 5 Ps. rooti and 3 Lu. saulensis. All were either developmental stages of a trypanosome or infections with monogenetic insect flagellates. A similar number of Lu. flaviscutellata dissected at the same time yielded infections of L. m. amazonensis.

Fresh mammalian blood meals, presumably from the bait animal, were seen in Lu. infraspinosa, Ps. rooti, Lu. saulensis, and Lu. monsturosa.

Discussion

In Panama, CHANIOTIS et al. (1971) noted that the sandfly population was usually much lower in the dry season and reached a peak just before the wettest month of the year. These same workers (CHANIOTIS et al., 1971a) concluded that the activity of the anthropophilic species was not totally in accordance with the specific or total sandfly population trends, which had been determined by catches from light and non-baited suction traps. WILLIAMS (1965) pointed out that different methods of collecting sandflies gave different estimates of the relative abundance of the different species, and that it was difficult to assess the significance of the differences between them. FAIRCHILD (1958) noted that although light traps yielded the largest number and variety of species, they did not give a true picture of the relative abundance. We are of a similar opinion: only very rarely, for instance, has Lu. flaviscutellata been collected in light traps or from tree trunks and this may be said for certain anthropophilic species. We feel that the discordant results observed in Panama might have been as much a reflection of the different
trapping methods as the effect of such undefined factors as "variation in physiological state of female flies" (Chaniotis et al., 1971a).

The highly localized distribution noted by us for Lu. flaviscutellata (Shaw and Lainson, 1968) was also noted for the species of sandflies caught in smaller numbers. Fairchild (1958), in discussing the habits of Panamanian sandflies, noted a similar patchy distribution, and remarked that the large catches of sandflies were exceedingly local, often being limited to one small area. The reasons for such phenomena are not at all clear, but we feel it may be something to do with the location of the trap in relation to breeding sites, because in such catches the proportion of males to females is always higher than normal.

In addition to the above mentioned localized distribution pattern, however, it is quite clear that Lu. flaviscutellata is more common in the wetter forests of the "igapo" type than in the drier forests of the "terra firme". Thus in October 1968 the average nightly catch in Catu was 5-3 times greater than in Utinga.

The seasonal fluctuations of Lu. flaviscutellata are clearly associated with rainfall (Figs. 1, 2) and it would seem that a monthly rainfall above 200 to 250 mm. is prejudicial to this species of sandfly. The Lu. flaviscutellata population, therefore, declines during the rainy season and steadily builds up during the dry season. In British Honduras, Disney (1968), using rodent-baited fly traps, noted that Lu. olmeca disappeared during the dry season and reappeared during the wet season. It would appear, at first sight, that Lu. olmeca differs from Lu. flaviscutellata in its seasonal fluctuations. In British Honduras, however, the rainfall did not exceed 160 mm. per month during the peak population period while in Belém the average monthly rainfall during the peak period was below 250 mm. From this comparison it would seem then that both are, in fact, affected similarly by rainfall.

All of the trypanosomatid infections that we have so far encountered in Lu. flaviscutellata have been L. m. amazonensis. In the small number of other species that we examined, however, we found relatively high infection rates of flagellates other than Leishmania. We feel that this is because Lu. flaviscutellata feeds almost exclusively on the rodents Proechimys and Oryzomys in the area of the present studies. To date we have found no trypanosome infections in Oryzomys: Proechimys is frequently infected with T. renjifoi (Deane 1961) but this trypanosome is of the subgenus Herpetosoma which are in general transmitted by fleas. The other species of sandflies would have presumably been found infected with Leishmania if the trypanosomal infections had been of rodents, as leishmaniasis is more common in these rodents than are trypanosome infections. To date, then, we have no evidence that any species of sandfly, other than Lu. flaviscutellata, is involved in the transmission of L. m. amazonensis.

In Utinga, Guimaraes et al. (1968) captured fewer infected O. capito in the middle of the year and we also noted a similar drop in the new infections in this same rodent during June and July in Catu and A.P.E.G. From this we conclude that the transmission rate is reduced during the previous months. We would emphasize, however, that although infected animals were found during these months they had been registered as being infected in previous months.

A number of factors influence the appearance of new infections of leishmaniasis in a wild mammal population, two of the most important being the seasonal fluctuations of the vector population and the influence of a defined breeding period of the mammalian reservoir. Although Oryzomys breeds throughout the year (Anon, 1964), more animals are born towards the beginning of the year. This coincides with the time when the Lu. flaviscutellata population is at its highest and one might expect to get a higher percentage of new infections during these months. This trend was observed in the Catu and
A.P.E.G. forests, as can be seen from Figure 3, where there was an indication of a slightly higher number of new infections during the first half of the year.

The coincidence of new infections of *L. m. amazonensis* in *Oryzomys* with the seasonal fluctuations of *Lu. flaviscutellata*, however, indicates that the major factor controlling the infection rate is the relative abundance of this sandfly. In both Catu and A.P.E.G. the populations of *Lu. flaviscutellata* fall to a minimum in April and May and the number of new rodent infections also fell to zero during the months of June and July. This drop in the number of new infections in these two months is possibly due to a reduced transmission rate following the fall in the sandfly population during the previous months. As the sandfly population increases towards the end of the year the transmission rises and the number of new infections goes up during the latter half of the year.

Using the number of trapped *Lu. flaviscutellata* as a guide, it seems that the critical number of sandflies is around 15 to 20 per night, as the number of newly infected rodents falls off markedly following periods of catches any lower than this. This had an interesting application in Utinga when we were searching for infected *Lu. flaviscutellata* during our earlier studies (LAINSON and SHAW, 1968; SHAW and LAINSON, 1968). The average number of flies in the trap that gave us positive flies was 19.7. In other traps that gave fewer numbers we were unable to find infected flies even though we dissected many hundreds. This does not necessarily mean that transmission has stopped, but that it is at such a low level that it is difficult to find either infected flies or rodents.

In comparing rodent enzootic leishmaniasis caused by *L. m. mexicana* in British Honduras and by *L. m. amazonensis* in the lower Amazon region of Brazil it is clear that a far greater sandfly population (*Lu. flaviscutellata*) is associated with the enzootic in Brazil than in British Honduras (*Lu. olmeca*). DISNEY (1968), for instance, records a maximum average of 7 flies per day while our maximum averages were in the order of 145 flies per day in Catu and 49 per day in Utinga. Under such conditions the infection rate of *L. m. mexicana* in *Ototylomys phyllotis* was 3.6% whereas the infection rate of *L. m. amazonensis* in *O. capito* was 7.7%. The infection rate of *L. m. mexicana* in *Lu. olmeca* varied from 0.3 to 0.9% (DISNEY, 1968) while in Belém the infection rate of *L. m. amazonensis* in *Lu. flaviscutellata* was approximately 0.9% (LAINSON and SHAW, 1968). These figures suggest that the ecological conditions in Belém are such that proportionately more flies are required to obtain similar transmission rates. From this we conclude that the ecology of enzootic rodent leishmaniasis in British Honduras and in the Belém region of Brazil are different. This may be related to such factors as relatively high mortality rates of the adult females or a different type of association between the vector and mammalian reservoir host.

**Summary**

The seasonal fluctuation of *Lu. flaviscutellata* is shown to be closely related to rainfall, the population of this species declining during the rainy season, between May and March, and increasing again during the dry season, reaching a peak towards December and January. A comparison of the numbers of *Lu. flaviscutellata* caught in different types of forest showed that this species is more common in the wetter forests of the "igapo" than in the drier "terra firme" forests, although similar seasonal fluctuations are noted in both types of forest. The close correlation between the numbers of *Lu. flaviscutellata* and the incidence of initial infections of *Leishmania mexicana amazonensis* in *Oryzomys capito* indicates that the major factor controlling the incidence of leishmaniasis is the number of sandflies.
REFERENCES


