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ORIGINAL ARTICLE

VALIDATION OF PHLEBOTOMINE CARDINAL TEMPERATURES AND HUMIDITIES IN THE REGION OF RIO ARINOS, MATO GROSSO, BRAZIL

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ABSTRACT

The purpose of this study was to validate the phlebotomine cardinal temperatures and humidities, reported by Campelo Júnior et al. (2014), using different collection data, obtained in a study on the number of these insects, captured in the Arinos region, in Nova Mutum, Mato Grosso, Brazil, according to spatial (100 m to 1000 m) and temporal (June 2011 to April 2012) variability. Phlebotomines were captured in the riparian forest, by means of 10 traps positioned approximately every 100 m, northwards from the road along the river bank, with samples obtained bimonthly during three consecutive nights for a period of 12 months. Average relative humidity and temperature during the periods when the traps remained at the collection points were measured using a digital thermometer-hygrometer. The phlebotomine sand fly fauna was highly diversified, presenting 31 species, of which *Lutzomyia antunesi* was the most prevalent (45.4%). *L. flaviscutellata, L. whitmani* and *L. umbratilis*, known vectors of *Leishmania* spp., were also found. There was a marked variation in the quantity of phlebotomines captured throughout the consecutive collection days, as each sampling was affected by different factors determining a different maximum value for the number of insects present in each situation, as demonstrated for temperature and air humidity.

KEY WORDS: Leishmaniasis; vector abundance; abiotic variables; Law of the Minimum.

INTRODUCTION

American tegumentary leishmaniasis (ATL) is reported in every Brazilian state and the Mid-West region is ranked third among the Brazilian regions for incidence and first for growth of the disease. In Mato Grosso, 100% of the municipalities have reported cases (Missawa et al., 2008).The disease is endemic in this state, with 21,000 cases reported between 2009 and 2017 (Brasil, 2018).

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The protozoa of leishmaniasis is transmitted to man and to other mammals by vector insects of the *Lutzomyia* genus, commonly known as sand fly, sand gnat, no-see-um, granny nipper, chitra or punkie (Fiocruz, 2014). All these common names are used in Brazil.

Ribeiro et al. (2007) reported that species of phlebotomine acting as vectors of ATL agents in Mato Grosso originate from several ecosystems.

Thies et al. (2013) captured the species *Lutzomyia antunesi, L. flaviscutellata, L. yuilli yuilli, L. ubiquitalis, L. umbratilis* and *L. whitmani*, species considered vectors of *Leishmania* spp., in a rural area of the municipality of Nova Mutum (MT), and the presence of *Leishmania infantum* DNA in *L. antunesi* led these authors to suggest the risk of visceral leishmaniasis (VL) being transmitted at the site.

At the beginning of the current decade four cases of cutaneous leishmaniasis were diagnosed at the leishmaniasis Referral Services in the Júlio Müller University Hospital/UFMT, in Cuiabá, MT. The patients all came from a wooded area on the banks of the Arinos River, in the town of Nova Mutum.

Rutledge & Ellenwood (1975), Zeledon et al. (1984), Salomón et al. (2002), Teodoro et al. (1993), Barata et al. (2004), Saraiva et al. (2006), Dias et al. (2007) and Reinhold-Castro et al. (2008), studied the effect of meteorological variables, which might explain seasonal variations in the occurrence of phlebotomine sand flies, but the results found in several of these studies revealed significant correlation, only in a few situations.

If the number of Phlebotominae at a given moment and environment depends on the availability of shelter, food, temperature, humidity and other variables. One alternative for evaluating the isolated effect of each of these variables would be to use a multiplicative mathematical model, in which the effect of each variable could be represented by a factor that varies from 0 to 1. The number of insects found in a certain situation, would be equal to the maximum value of the number of insects that occur, when all the conditions are favourable, multiplied by the factors that represent the other variables, reaching the maximum value as each factor moves away from the unit, thus reducing the number of insects to the limit imposed on their occurrence, by the environment.

The Law of the Minimum establishes that if one biological variable is affected by several environmental variables, the increase in that variable is limited by the environmental variable which presents the lowest availability. Therefore, quantification of the Law of the Minimum has been applied in several ways, for example, as used by Xu (1996) and Wang & Engel (1998).

The analysis of relationships between any variables is performed by modelling or by empiric statistical approach. The Pearson correlation coefficient is a preliminary empiric statistical parameter for analysing unknown relationships between variables. The modelling approach requires validation with different data from that used by parametrization and model creation.

Considering data collected over the course of three consecutive years, Campelo Júnior et al. (2014) used the Wang & Engel (1998) mathematical model to determine the minimum, maximum and optimum temperature and relative air humidity values for the occurrence of phlebotomine sand flies at the Universidade Federal de Mato Grosso campus, in Cuiabá.

The purpose of this study was to validate the phlebotomine cardinal temperatures and humidities reported by Campelo Júnior et al. (2014), using other collection data obtained in a study of the spatial (100 to 1000 m) and temporal (June 2011 to April 2012) variability in the number of these insects, captured in the Arinos region, in Nova Mutum, Mato Grosso, Brazil.

MATERIAL AND METHODS

The study was conducted on the right bank of the Arinos river, on the municipal order of Nova Mutum and São José do Rio Claro, Mato Grosso State.

Ten points were selected for the placement of traps, numbered in sequential order from 1 to 10, positioned approximately every 100 m northwards, 50 m towards the river, from the edge of the woods, located next to the bridge over the highway that connects the two municipalities. The site consists basically of primary, native, closed riparian forest of the Brazilian savannah; a site that can be used for both bathing and fishing, common leisure activities for local residents.

The sand fly captures were carried out bimonthly, on alternate months, during a one-year period (June, August, October and December 2011) and (February and April 2012), for three consecutive nights per month, totalling six capture campaigns. CDC (Center on Disease Control) light traps (Sudia & Chamberlain, 1962), powered by 12 volt batteries, were used to capture the Phlebotominae.

The traps were set up at dusk and were left on continuously from 17:00 to 07:00 the next morning, approximately 1.5 metres above ground. One trap was installed at each collection point.

The insects were duly packed and transported in an isothermal container to the Municipal Health Department of Nova Mutum, for the other procedures. The insects were euthanized with ethyl acetate (PA).

All the specimens were triaged and packed in test tubes containing a 70% alcohol solution for subsequent preparation, mounting and identification. Species identification was performed using taxonomic keys and comparison with specimens kept in the Insect Laboratory collection of the Mato Grosso State Health Department. Classification followed that proposed by Young & Duncan (1994).

The phlebotomines were clarified by means of immersion in a Potassium Hydroxide solution (10%) for 3 hours, followed by acetic acid (10%) for 20 minutes, and three series of washing with distilled water for 15 minutes each. They were then immersed in lacto-phenol for 24 hours, and subsequently mounted on glass slides in Berlese fluids in accordance to a modified version of Langeron's method (1949).

The contrasts between insect numbers in species, sex, capture sites, time of the year and consecutive collection days were evaluated by the Chi-square test.

To verify the influence of the climatic variable on the sand fly population, data for temperature (ºC) and relative air humidity (%) were used, relative to the period of June 2011 to April 2012. The abiotic data regarding temperature and average relative humidity during the times when the traps remained at the insect collection sites were determined by the maximum and minimum readings obtained with a digital TFA 7429 thermometer-hygrometer, installed in a shelter, approximately 1.5 metres above ground, located at collection point number 1, from 17:00 h to 07:00 h the next morning.

The effect of the abiotic variables X_i considered (temperature, T, in ºC and relative humidity, UR, in %) on the number of insect captures (N) was evaluated according to the methodology used by Campelo Júnior et al. (2014), where this number is affected by several variables or factors, each of which, may be in a condition that independently influences the number of insects. Therefore, there is a maximum number of insects captured (N_{max}) for each condition or value of those variables. The actual number observed may be equal to or less than N_{max} conditioned by the studied variable, since the other intervening factors may impose more limitations. The effect of an isolated variable should be determined by taking into account only the maximum value that it conditions. As the maximum number conditioned by a variable, such as temperature, varies in function of the temperature, there is a record number of insects (N_{rec}) that can be captured when the temperature is most favourable (optimum temperature). When the abiotic variable takes on values less than the minimum value, under the required limit for the occurrence of the insect in the environment, or greater than the corresponding maximum value, the number of insects captured is nil. Alternatively, the Pearson correlation coefficient was applied to analyse the relationship between the number of captured insects and temperature and relative humidity.

RESULTS

Over the course of the study 3,743 sand flies were caught. Males numbering 1,005 specimens (27%) and females 2,738 (73%). Fauna consisted of 31 species, one of which belonging to the *Brumptomyia genus: B. brumpti* (França & Parrot, 1921) and 30 to the genus *Lutzomyia*, of which the most abundant were *L. antunesi* (Coutinho, 1939) totalling 45%, *L. saulensis* (Floch & Abonnenc, 1944) totalling 21%, *L. walkeri* (Newstead, 1914) totalling 13% and *L. flaviscutellata* (Mangabeira, 1942) totalling 6%. The other species totalled less than 1.5% each (Table). The phlebotomine fauna of Nova Mutum, Mato Grosso, proved highly deversified, with 31 different species, of which *L. antunesi* was the most prevalent, accounting for 45.4% of the specimens captured. *L. flaviscutellata, L. whitmani* and *L. umbratilis* were the medically important species found in the study. The identification of the species was performed according to the procedures valid at the time of data collection, without the further taxonomic contribution by Galati & Ovallos (2012), Sábio et al. (2014) and Godoy & Galati (2016).

The only collection failures were noted in trap 8, on June 6, 2011, in trap 6 on October 17, 2011, and in trap 9 on December 12, 2011. The total number of Phlebotominae captured in a single day varied from 75, on June 7, 2011, to 625, on December 14, 2011. The lowest variation between the three consecutive collection nights occurred in the second bimester of the sampling (collections 4, 5 and 6). In the third bimester (collections 7, 8 and 9), in the fourth bimester (collections 10, 11 and 12) and in the final bimester (collections 16, 17 and 18), the variation in the three consecutive collection nights was practically the same as the variation found over the entire study period. Most collection nights produced less than 200 phlebotomines/night (Figure 1).

The site where the highest number of phlebotomines was captured throughout the entire collection period corresponded to the penultimate point (trap 9), followed by the final point (trap 10) and the first (trap 1), which was closest to the bridge on the edge of the forest.

The error bars in Figure 2 correspond to the variation amplitude at each site and indicate that, in every location, there were days with less than 15 sand flies collected. However, the maximum number of sand flies captured in a single night was less than 50 at only two sites. In traps 1, 9 and 10, the number of insects collected in one night was greater than 120, with the aggravating factor of these records occurring at different times of the year.

Specimens of *L. antunesi, L. dasypodogeton, L. flaviscutellata, L. furcata, L. llanosmartinsi, L. saulensis* and *L. walkeri* were caught at all the CDC trap points. However, most of the species did not maintain the same distribution pattern, being present at some points and absent at others, such as for example, the species *L. yuilli yuilli* which appeared in traps 1, 3, 5, 8, 9 and 10.

Table. Total number of Phlebotominae captured in 10 traps (A1 to A10), installed along the right bank of the River Arinos, between Nova Mutum and São José do Rio Claro, MT, in six two-monthly sampling campaigns, of three consecutive days, in the period 06/06/2011 to 26/04/2012, in accordance with species and sex (F=female, M=male), in the municipality of Nova Mutum – MT.

Species	A1		A2		A3		A4		A5		A6		A7		A8		A9		A10		
	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	Total
B. brumpti	0	0	0	1	1	1	3	0	3	1	1	1	1	0	0	0	3	0	2	2	20
L. antunesi	150	153	121	65	63	32	56	14	127	42	78	26	53	23	138	31	338	28	142	21	1701
L. aragoi	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	1	0	1	0	4
L. ayrozai	2	0	3	0	0	0	2	0	4	0	7	0	2	0	2	0	5	0	6	0	33
L. begonae	0	0	2	0	1	0	1	0	5	0	0	0	1	0	3	0	7	0	8	0	28
L. bourrouli	0	0	1	0	0	0	0	0	2	1	0	0	0	0	2	0	1	0	0	0	7
L. chagasi	1	0	0	0	1	0	1	0	0	0	2	0	1	0	1	0	3	0	5	0	15
L. claustrei	5	26	3	0	1	0	0	2	1	0	0	0	1	0	2	9	1	1	0	2	34
L. complexa	0	0	0	0	0	0	0	0	1	0	1	1	1	0	1	0	0	0	0	0	5
L. dasypodogeton	1	0	0	1	2	0	1	0	1	2	0	3	2	0	1	0	1	0	3	0	18
L. davisi	0	0	0	0	14	2	1	1	0	0	0	0	0	0	0	1	0	0	2	0	21
L. flaviscutellata	28	14	10	11	3	0	29	21	18	19	9	2	8	2	19	6	8	10	3	10	230
L. furcata	2	1	1	0	1	0	3	0	8	0	3	0	1	1	5	0	9	0	1	0	36
L. hermanlenti	0	0	0	2	0	0	0	0	2	4	0	2	0	0	1	0	4	0	1	1	17
L. llanosmartinsi	3	0	2	0	7	1	2	0	1	0	8	1	3	0	3	0	9	0	8	0	48
L. lenti	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
L. longipennis	0	3	0	1	0	0	0	0	5	0	2	1	1	0	1	0	0	0	0	1	15
L. octavioi	0	2	0	0	0	0	0	1	0	0	0	2	0	0	0	0	0	0	0	0	5

Table. Total number of Phlebotominae captured in 10 traps (A1 to A10), installed along the right bank of the River Arinos, between Nova Mutum and São José do Rio Claro, MT, in six two-monthly sampling campaigns, of three consecutive days, in the period 06/06/2011 to 26/04/2012, in accordance with species and sex (F=female, M=male), in the municipality of Nova Mutum – MT.

Species	A1		A2		A3		A4		A5		A6		A7		A8		A9		A10		
	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	Total
L. punctigeniculata	2	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	3
L. runoides	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0	0	3
L. sallesi	0	0	0	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
L. saulensis	74	14	65	20	51	14	65	7	137	14	44	5	39	5	58	3	75	6	69	5	770
L. shannoni	3	0	4	0	3	0	2	0	2	0	4	0	0	0	8	0	3	0	5	0	34
L. shawi	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
L. sordelli	2	0	1	0	0	0	1	0	2	0	1	1	0	0	2	1	7	0	0	1	19
L. spp.	3	1	1	3	1	1	0	0	1	2	1	1	1	0	0	0	3	1	5	0	25
L. ubiquitalis	21	37	1	2	0	1	1	4	7	13	5	9	3	5	4	11	0	0	1	1	126
L. umbratilis	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1
L. walkeri	38	30	26	18	17	11	29	15	41	17	32	22	13	8	22	43	42	37	22	11	494
l.whitmani	1	2	0	1	0	0	0	0	0	2	0	0	0	0	0	1	0	0	0	0	7
l.yuilli yuilli	3	0	0	0	2	0	0	0	4	0	0	0	0	0	2	0	3	2	3	0	19
Total	339	263	242	126	168	65	198	65	376	117	199	77	131	45	275	106	523	86	287	55	3743

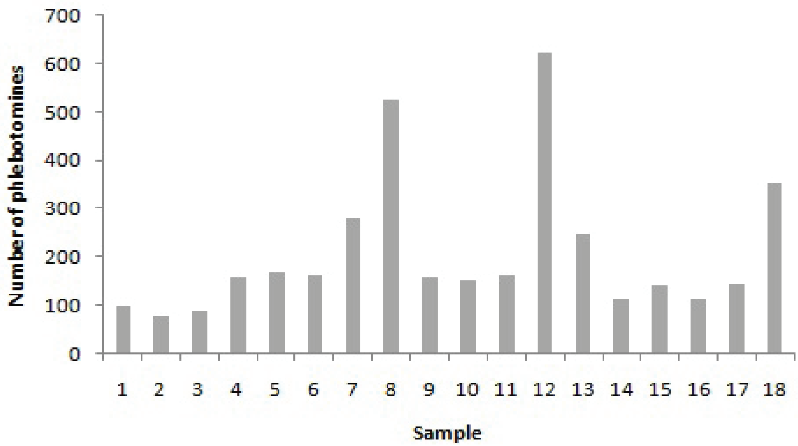

Figure 1. Number of Phlebotominae captured, with no distinction of species or sex, at each collection site, in 10 traps placed along the right bank of the Rio Arinos, in six two-monthly sampling campaigns on three consecutive days, between 06/06/2011 and 26/04/2012. (The columns represent mean values over the course of the study).

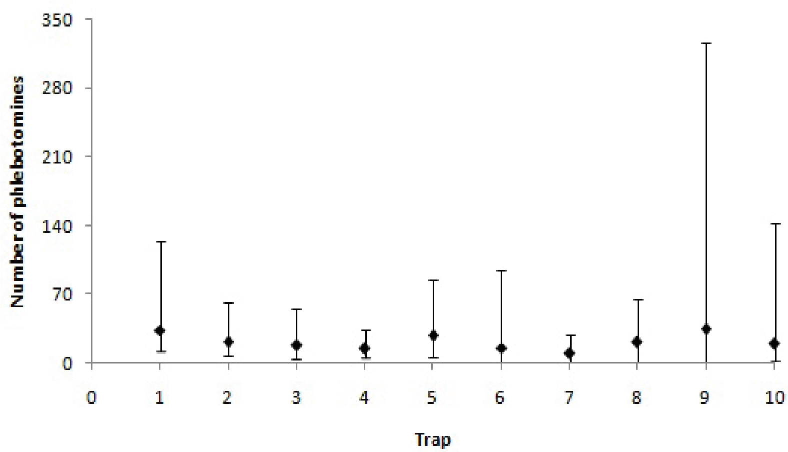

Figure 2. Total number of captured Phlebotominae, with no distinction of species, sex or trap location, in 10 traps placed along the Rio Arino banks, at the municipal border of Nova Mutum and São José do Rio Claro, MT, in six two-monthly sampling campaigns conducted on three consecutive days, in the period 06/06/2011 to 26/04/2012.

L. antunesis stood out for its presence in every collection month and, except for October, always predominated during the months studied.

Upon analysis of the total values observed over the course of the study, by means of the Chi-square test, significant differences ($p < 0.01$) were found between sex (17 df), capture sites (9 df), time of the year (5 df), and consecutive collection days (17 df). The one by one comparisons of traps, for the five most abundant species (4 df),was the following: Only in trap 4 the numbers of these five species was different from the numbers in all the other traps; trap 9 was different from all the others, except trap 10; trap 4 was different from all the others, except traps 3 and 7, while trap 6 was different from all the others, except traps 7 and 8; the highest frequency of no significant differences was between neighbouring traps. Therefore, no spatial variation pattern was detected.

The variation between the three consecutive collection days, observed in Figure 1, as well as the variation amplitude at each site and at all the sites, observed in Figure 2, indicate that conditions favourable to the occurrence of phlebotomines varied abruptly, from one day to the next, regardless of the time of year in which the collection was performed.

In Figures 3 and 4, the symbol x represents the number of captured insects of each species, in each of the 10 traps, in each of the 18 samplings (three consecutive nights in six bimesters), according to the average temperature and relative humidity values throughout the time in which the traps remained at the collection sites, on each day. The (max) curves correspond to the maximum number established by applying Wang & Engel's parametrization model (1998), considering each abiotic variable independently, with the minimum, maximum and optimum values of the abiotic variables found by Campelo Júnior et al. (2014). The results obtained in this study are within the limits for temperature and humidity established in the captures executed in Cuiabá (Campelo Júnior et al., 2014).

The Pearson correlation coefficient between total insect number and temperature and total insect number and relative humidity were -0,09 ($p = 0.71$) and 0,28 ($p = 0.26$), respectively.

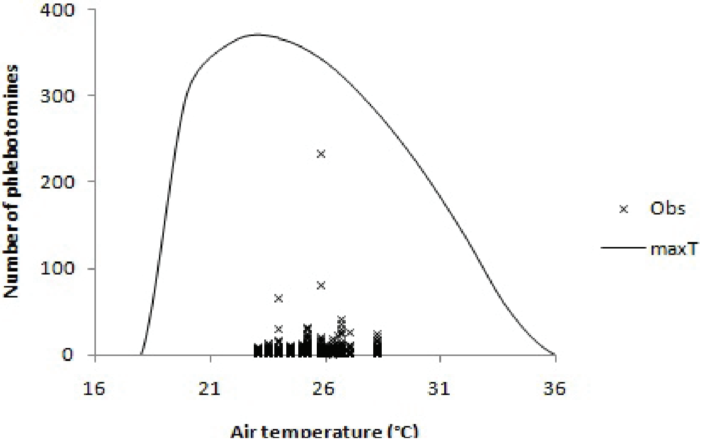

Figure 3. Number of Phlebotominae captured (obs), according to average air temperature measured at the collection time on the right bank of the Rio Arinos, at the municipal between of Nova Mutum and São José do Rio Claro, in the period between 06/06/2011 and 26/04/2012, maximum number (maxT) established by the mathematical model for quantifying the effect of average temperature in the same conditions, considering Campelo Júnior et al. (2014).

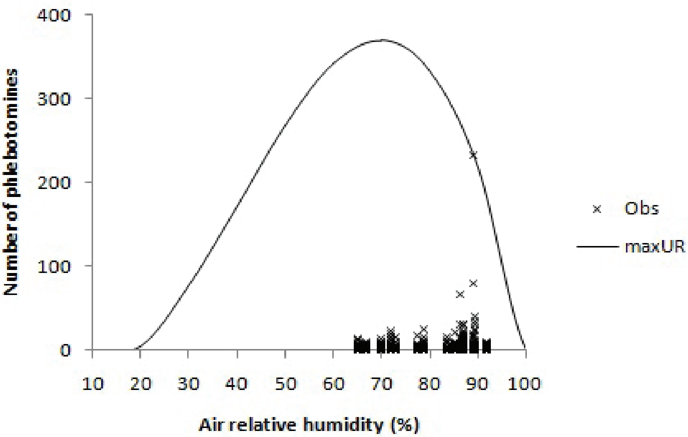

Figure 4. Number of Phlebotominae captured (obs), according to average relative air humidity measured at the collection time on the right bank of the Rio Arinos, at the municipal limits between Nova Mutum and São José do Rio Claro, in the period between 06/06/2011 and 26/04/2012, and maximum number (maxUR) established by the mathematical model for quantifying the effect of average relative humidity under the same conditions (Campelo Júnior et al., 2014).

DISCUSSION

Other studies conducted in the state of Mato Grosso have revealed a similar quantity of phlebotomine sand flies to those observed at the study site, distributed in different kinds of areas, and all the species found in this study had been previously detected in Mato Grosso (Maciel & Missawa, 2007 and 2009; Amaral et al., 2011; Alves et al., 2012). Azevedo et al. (2011), in a study conducted in a tropical forest fragment in São Luis, Maranhão State, on the existence of differences between species at the edge and in the middle of the forest, found *L. antunesi* prevalent in their sampling (68.9%), a higher result to that found in this study, however the authors report that one of the fragments studied contributed 71% of the *L. antunesi* specimens. Azevedo et al. (2011) found that the species *L. flaviscutellata* was among the four most abundant, also corroborating the results noted here. Vásquez-Trujillo et al. (2008) in a study developed in a rural area at a site in Villavicencio, Colombia, found *L. antunesi* as the most abundant phlebotomine sand fly (75.6%), followed by *L. walkeri* (19.2%).

In this study significant differences were also found regarding the collection points, probably due to the diversity of factors that affect the occurrence of these insects, which can lead to differences, even at distances of less than 100 m along the same river, in the same type of ecotope and similar spatial scale. In a previous study, in a similar analysis to those performed in several other studies, Thies (2012) found that there was no correlation between temperature, relative humidity and rainfall and the number of phlebotomines caught, but reported that the highest phlebotomine sand fly capture count occurred in the rainy season (October, December), when both relative humidity and temperature were higher. In the lower Amazonian region, Shaw & Lainson (1972) detected an increase in the population of sand flies during the wet season and a decrease during the dry season. Galati et al. (1996) found that the *L. whitmani* population in Corguinho, Mato Grosso do Sul, was lower in the warmer wetter months and peaked during the cooler drier months.

Throughout the whole study the numbers of captured insects of both sexes, of all species and in all the sites, remained less than the maximum predicted value and are identified in Figure 3 and Figure 4 as the points located below the max curves. These points below the max curves mean the captures made when other biotic or abiotic factors, such as availability of food, were more limiting than the air temperature or humidity for the occurrence of Phlebotominae, in accordance with the Law of the Minimum. This interpretation extends to all the points in Figure 3 and in Figure 4 that are below the (max) line, at any temperature, between 22.5ºC and 30.0ºC, and at any relative humidity between 20% and 93%. The variations found in all the aspects analysed confirm the results published in the literature, which indicate that the quantity of captured insects is influenced by various factors. As in other cases of biological variables, the most limiting likely factor establishes a maximum value for the number of insects present in each situation.

The concentrated results seen in the lower central section of Figure 3, relative to temperature, and in the lower right section of Figure 4, relative to humidity, are probably due to the variation ranges of temperature and relative humidity within which the current results were obtained. Whereas in this study, the field data was collected at temperatures varying from 23.1ºC to 28.3ºC and relative humidity from 65% to 92%, Campelo Júnior et al. (2014) conducted collections from 2008 to 2011 on the UFMT campus in Cuiabá, when the temperature varied from 33.9ºC to 18.0ºC and the relative air humidity varied from 93% to 21%. As the study conditions in Cuiabá were broader, especially in relation to the lowest temperature and humidity values measured, no temperatures below 23.1ºC and no humidity levels below 65% were recorded in the current case, possibly because temperature and humidity vary less in the forested area of the Arinos river bank.

The notion that there could be minimum and maximum limiting factors, and an optimal level for each factor that influences the occurrence of phlebotomine sand flies, was propounded by Rutledge & Ellenwood (1975), when they considered that moderate levels of rainfall were propitious for insects, but excessive levels would be detrimental as breeding grounds are destroyed and pupae in the soil are killed.

High concentrations of phlebotomine sand flies have been found in warm and humid conditions (Salomón et al., 2002), yet also in drier conditions, as observed by Zeledon et al. (1984). Barata et al. (2004) found a significant correlation between the number of Phlebotominae captured and rainfall and humidity, whereas temperature did not have a significant effect on the number of insects. Saraiva et al. (2006) found a positive correlation between temperature elevation and the number of Phlebotominae collected at every sampling point in their study. For Dias et al. (2007), temperature, humidity and rainfall do not constitute determining factors regarding the number of adult phlebotomines. Teodoro et al. (1993) and Reinhold-Castro et al. (2008) observed that the number of phlebotomine sand flies was greater when rainfall and temperature were higher. As well as the different species and the different environments and dates, the apparent contradictions between the other studies mentioned might be explained in light of the fact that the effects have multiple causes, each of which with relations that may cause an increase or reduction, due to the variation ranges of the factors involved.

The variation in the quantity of Phlebotominae captured in the sampling campaigns, indicates that favourable conditions for the occurrence of these insects can vary dramatically from one day to the next, regardless of the time of year. They can also vary from one collection trap to another, and the confirmed results previously reported in the literature, indicate that the quantity of sand flies captured is influenced by various factors, as in other cases of biological variables, when the effect of the most limiting factor establishes a maximum value for the number of insects present in each situation (Rutledge & Ellenwood, 1975; Zeledon et al., 1984; Salomón et al., 2002; Teodoro et al., 1993; Barata et al., 2004; Saraiva et al., 2006; Dias et al., 2007; Reinhold-Castro et al., 2008).

The results showed that there were different species in the studied area. Each species should present its own cardinal humidity and temperature. However, phylogenetic attributes could be both the cause and consequence of the different humidity and temperature limits, showing some similarities within genera and subfamilies. Therefore, these minimum, optimum and maximum values are merely a first approach to Phlebotominae requirements (Campelo Júnior et al., 2014).

Considering the Pearson correlation coefficient results, the analyzes using the Law of minimum principle and the Wang & Engel mathematical model (1998), with cardinal temperature and relative humidity determination, proved a better approach to describe the complexity of results found than the correlation analysis.

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