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Seasonal variation of sand fly populations in Kala-azar endemic areas of the Malda district, West Bengal, India

Ashif Ali Sardar M. Sc [a], Moytrey Chatterjee M. Sc, Ph. D [a,*], Kingsuk Jana M. Sc [a], Pabitra Saha M. Sc, Ph. D [a,b], Ardhendu Kumar Maji M. Sc, Ph. D [a], Subhasish Kamal Guha M. D [c], Pratip Kumar Kundu M. D [a]

[a] Department of Microbiology, Calcutta School of Tropical Medicine, Kolkata, West Bengal, India
[b] Department of Zoology, A. P. C. Roy Govt. College, Himachal Bihar, Matigara, Siliguri, West Bengal, India
[c] Department of Tropical Medicine, Calcutta School of Tropical Medicine, Kolkata, West Bengal, India

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ABSTRACT

Vector control is one of the main aspects to reach the target of eliminating visceral leishmaniasis from Indian sub-continent as set by the World Health Organisation. Data on different aspects of vector like ecology, behaviour, population dynamics and their association with environmental factors are very important for formulating an effective vector control strategy. The present work was designed to study the species abundance and impact of environmental factors on population dynamics of vector *P. argentipes* in a visceral leishmaniasis endemic area of Malda district, West Bengal. Adult sand flies were collected using light traps and mouth aspirators from twelve kala-azar affected villages of Habibpur block of Malda district, on a monthly basis from January to December, 2018. Morphological and molecular methods were used for species identification. Population dynamics were assessed by man hour density and per night per trap collection. Data were analysed using SPSS software to determine the impact of environmental factors on vector population. *P. argentipes* was found to the predominant species and prevalent throughout the year. A significantly higher number of sand flies were collected from cattle sheds than human dwellings and peri-domestic vegetation. A portion of the *P. argentipes* population was exophilic and exophagic as evidenced by their collection from peri-domestic vegetation. The highest population density was recorded during April to September. Population dynamics were mostly influenced by average temperature along humidity and rain fall. Resting behaviour of sand flies was not restricted to the lower portion of the wall but equally distributed throughout the wall and ceiling. Programme officials should consider management of outdoor populations of the sand flies and timings of indoor residual spray for chemical control purpose.

1. Introduction

Visceral leishmaniasis (VL) or Kala-azar (KA) is a vector borne parasitic disease and is mostly fatal if not treated properly (Bern et al., 2005). It is the second most deadly parasitic diseases next to malaria (Desjeux, 2001a,b). Globally, an estimated 50,000–90,000 VL cases reported each year and 95% of global burden is contributed by India, Bangladesh, Sudan, Ethiopia, and Brazil (Alvar et al., 2012; WHO, 2019).

VL is caused by the protozoan parasite *Leishmania donovani,* which is transmitted by the female phlebotomine sand flies. Sand flies are tiny dipteran insects under the family: Psychodidae. Among 800 known species of phlebotomine sand fly (Young and Duncan, 1994) several species of two genera have been proven as vector of leishmaniasis: *Phlebotomus* in the Old World and *Lutzomyia* in the New World (Alexander and Young, 1992; Killick-Kendrick, 1999).

In Indian subcontinent VL is anthroponotic having no vertebrate host reported so far other than human (Dinesh et al., 2009; Swaminath et al., 1942), *L donovani* is the only parasite and *P. argentipes* is the only known vector (Sharma et al., 2006; Rijal et al., 2006). Considering these facts, three adjoining countries, India, Bangladesh and Nepal initiated a VL elimination programme in 2005 supported by the World Health Organisation (WHO) with a target to eliminate VL from this part of the world by 2015 (WHO, 2005). This initiative was revised and two more coun-

* Corresponding author.
E-mail addresses: ashifbappa786@gmail.com (A.A. Sardar); moytreychatterjee@yahoo.in (M. Chatterjee); kingsukjana11@gmail.com (K. Jana); pabitra.saha82@gmail.com (P. Saha); maji_ardhendu@yahoo.com (A.K. Maji); drskguha@gmail.com (S.K. Guha); pratipkundu1955@gmail.com (P.K. Kundu)

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tries, Bhutan and Thailand were included with a new goal of eliminating VL by 2020 (WHO, 2014). Integrated vector control is one of the main aspects to reach the target by reducing the transmission of parasite with limited man-vector contact (WHO, 2005). For formulating an effective vector control strategy, data on several aspects of sand fly ecology – species abundance, feeding and resting behaviour, population dynamics, impact of environmental factors (temperature, rain fall and humidity) on population dynamics, and susceptibility to different insecticides are very important (Warburg and Faiman, 2011).

There are several reports regarding species abundance and distribution of sand flies from different parts of India (Dhanda and Modi, 1971; Kumar et al., 1992; Kaul, 1993; Ilango et al., 1994; Sharma et al., 2005; Srinivasan and Jambulingam, 2011; Srinivasan et al., 2013; Poche et al., 2018) but such data from West Bengal is very limited. The influence of environmental factors such as temperature, rainfall, relative humidity on population dynamics of sand fly have been reported (Srinivasan et al., 2013; Srinivasana et al., 2015; Poche et al., 2018) from different VL endemic areas of India. But no such data are available particularly from eastern region of India including West Bengal. Morphological identification of sand flies is very difficult and requires skilled entomologists. Recently several authors used molecular tools based on characterization of 18S rRNA by PCR-RFLP (Tiwary et al., 2012; Poche et al., 2018). Now it is easier for identification of sand flies by using established molecular methods. Only a few reports have been published regarding use of these approaches for species identification, of wild caught sand flies. The present work was designed to study the species abundance, population dynamics and the role of environmental factors on sand fly populations in VL endemic areas of Malda district, West Bengal.

2. Materials and methods

2.1. Study area

Our study was conducted in 12 villages in the Habibpur block of the Malda district, West Bengal (Latitude 88.2353°E, Longitude 25.0156°N). The villages were selected on the basis of reported VL and PKDL cases over the last 5 years. The study was conducted during January, 2018 to December, 2018. The geographical location of study villages are given in Table 1.

2.2. Sand fly collection

Before initiation of this study, an informational meeting was held with the villagers in the presence of state and district health officials and local health and administrative personnel. The objectives of the study were explained and individuals were requested to assist the project staff during the study period. Permission was obtained from the owners of private houses before collection of sand flies. The present study was not associated with any endangered and protected species. The study protocol was approved by the Institutional Ethics Committee of Calcutta School of Tropical Medicine, Kolkata, India. Sand flies were collected by two methods.

2.2.1. Light trap collection

CDC light traps with standard collection cup assemblies (Model 1012 miniature Incandescent Light Trap, John W. Hock, USA) were used for sand fly collection in the study area. CDC traps were placed in human dwellings, cattle sheds and peri-domestic vegetation (small shrubs, hurbs, and seasonal weeds) to estimate distribution of sand fly in different habitats of the study areas. Six light traps were placed in each village every month: two each in human habitation, cattle sheds and peri-domestic vegetation. Such collections were made during first two weeks of every months cyclically from each site. Traps were positioned about 1 m above the ground (Poche et al., 2011) and operated with 12 V batteries, remained activated between 05:00pm (previous day) and 6:30am (next day). The location of each light trap was numbered uniquely and collected sand flies were recorded date wise in a data sheet.

2.2.2. Aspirator collection

Indoor resting sand flies were collected using hand-held mouth aspirators and flash lights from human dwellings and cattle sheds. Sand flies were collected for 10 min / house during early morning (5:00am to 7:00am) from 20 houses of each village, once per month from January, 2018 to December, 2018 by three trained insect collectors. Weather data including maximum and minimum temperature (°C) and relative humidity (%) were recorded on the day of collection using a digital thermometer and hygrometer. The records of monthly average precipitation were collected from Website (http://www.timeanddate.com/Malda).

2.3. Identification of collected sand flies

Sand flies captured by light traps were placed in glass jar containing chloroform-soaked cotton balls and separated with the help of fine brush from other insects. Sand flies were counted and identified as to species on the basis of morphological features using a standard taxonomic key (Lewis, 1982) under a stereoscopic microscope on the day of collection. The sand flies were

Table 1
Geographical position of study villages.

District/Block	GPs	Sub-centre	Villages	Latitude (°E)	Longitude (°N)
MALDA/HABIBPUR	AKTAIL	Kharibari	Palasdanga	25.0405	88.3603
		Kendpukur	Rajarampur 2	25.0592	88.3314
		Binodpur	Patharbati	25.0938	88.3555
		Tapsahar	Narsinghabati	25.088	88.3652
	DHUMPUR	Dhumpur	Guinagar	24.9643	88.3057
		Langalbhanga	Khanpur Palasbona	25.5218	88.3494
		Fakirakandor	Sadapur	24.9849	88.2935
	KANTURKA	Laxmipur	Laxmipur	25.0386	88.3251
		Mirjapur	Muraraipur Jamdanga	25.1192	88.3761
		Nimbari	Gopalpur	25.0154	88.3664

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grouped into males and females on the basis of their morphological characteristics in the anal region.

For molecular speciation, identification 500 were morphologically identified (250 *P. argentipes* and 250 *P. papatasi*) from different study sites and were stored individually in 70% ethanol.

2.4. Molecular identification

DNA of sand flies stored in ethanol was extracted individually using QIAamp DNeasy mini kit (QIAGEN, Germany) according to manufacturer instructions. A PCR-RFLP method was adopted for species confirmation using the stored 18S rRNA encoding sequence as described earlier (Tiwary et al., 2012). In brief, the following forward and reverse primers were used for amplification of 18S rRNA encoding sequence: 5′-TAGTGAAACCGCAAAAGGCTCAG-3′ (Forward) and 5′-CTCGGATGTGAGTCCTGTATTGT-3′ (Reverse). PCR conditions were optimized as, initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 40 s, extension at 72 °C for 30 s, and a final extension at 72 °C for 10 min. The amplified PCR products were subjected to restriction digestion with *Hinf I* (recognition site GANTC; time saver, New England Bio labs, UK) at 37 °C for 10 min to discriminate between the species of sand flies. The digested products were electrophoresed on a 2% (w/v) agarose gel and stained with ethidium-bromide. A single band of 121 bp was considered as *P. argentipes* and two bands of 109 bp and 319 bp for *P. papatasi*. For further confirmation 15 PCR products were sequenced in both directions using forward and reverse primers. The sequences were aligned with reference strain of *P. argentipes* (GenBank no. AJ244360.1) and *P. papatasi* (GenBank no. AJ244414.1) using pairwise alignment tool.

2.5. Data analysis

The number of sand flies collected using CDC light traps and mouth aspirators were entered into an excel sheet according to the month of collection, species, sex, and habitat. Man hour density (MHD) for the mouth aspirator collected sand flies was calculated as: MHD = total number of sand flies collected / (total time spend x number of insect collector). Per trap per night collection was calculated as: total no. of sand flies captured / no. of trap used. The habitat wise distribution of sand flies (human dwellings, cattle shed, pre-domestic vegetation) was analysed by Kruskal-Wallis Test. The impact of environmental factors (temperature, humidity, rainfall) on population dynamics of sand fly were determined using Spearman's correlation analysis using software SPSS (version 21.0).

3. Results

3.1. Demography of the study villages

The study villages were about 40–45 km from Malda town. Villages were mainly inhabited by tribal populations with a low socio-economic status. The majority of the houses were constructed of mud walls and floors with poor ventilation, making the rooms dark during daytime. Very few houses were made of brick and cement. Agriculture was the main source of earning and some of them are migratory workers. Cattle rearing was a common practice. During the night, cattle were kept within the same house adjoining the human living rooms. Sometimes cattle were kept on the ground floor and first floor of the same house was used for human dwelling. During the day time cattle were kept in the courtyard adjoining to the human dwellings. The study villages were surrounded by fertile agricultural crops land and thick vegetation (trees and bushes such as *Magnifera indica, Azadirachta* sp, *Borassus* sp., *Phoenix* sp., *Ocimum* sp., *Lantana* sp., *Bambusa* sp., etc.) making the human habitation shady throughout the year.

The climatic conditions in the study area fluctuated seasonally. The period between March and June was summer, from July to October received monsoon rains. The months between November to February were relatively cool. The highest average temperature was recorded as 30 °C during the months of March to September, which gradually declined towards both January and December. The average maximum humidity (70–100%) was recorded during the month of April to September. The maximum rainfall was recorded during the month of May to September (Table 2).

3.2. Sand fly collection and species identification

During 12 months of the study period a total of 51,864 sand flies were collected by both the collection methods used, among which 14,129 were males, and 37,555 were female with the ratio of 1:2.66 (Table 3A and B). The month-wise male female ratio of collected sand flies is given in Fig. 1. Of the female sand flies, 59.48% (22,337) were unfed and 40.52 (15,218) were blood feed. Among the collected 51,864 sand flies 40,421 (77.9%) were identified as *P. argentipes* and 11,443 (22.1%) as *P. papatasi.* The monthly distribution of the two species is given in Fig. 2, and Table 3A and B. Molecular identification tools like PCR,-RFLP and DNA sequenc-

Table 2
Monthly average rainfall, humidity, and temperature (C°).

Months	Max. Temp.	Min. Temp.	Avg. Temp.	Max. Humidity	Min Humidity	Avg. Humidity	Avg. Rain
JAN	26	12	18	95	27	68	5.2
FEB	27	16	21	67	31	67	12.6
MAR	32	20	26	62	42	62	6
APR	33	23	28	70	51	70	77.9
MAY	36	22	30	98	55	86	140.6
JUN	33	27	30	81	59	81	314.8
JUL	36	20	30	100	56	82	192.2
AUG	33	27	30	84	50	84	258.1
SEP	35	25	30	97	53	79	191.8
OCT	30	16	24	97	30	68	92.1
NOV	30	19	24	74	36	74	3.1
DEC	28	15	21	94	36	68	1.6
MEAN	**31.58**	**20.16**	**26**	**84.91**	**43.83**	**74.08**	**108**
SD	**±3.39**	**±4.83**	**±4.32**	**±13.74**	**±11.44**	**±7.97**	**±110.38**
RANGE	**26–36**	**12–27**	**18–30**	**62–100**	**27–59**	**62–86**	**1.6–314.8**

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Table 3A
Month wise collection of *P. argentipes* from different habitats in Kala azar endemic areas of Malda, West Bengal.

Collection methods		Light trap collection									Aspirator collection					
Habitat		Human dwelling			Cattle shed			Peri-domestic vegetation			Human dwelling			Cattle shed		
		T	M	F	T	M	F	T	M	F	T	M	F	T	M	F
Months of collection	Jan	2	0	2	79	15	64	0	0	0	22	4	18	468	90	378
	Feb	9	3	6	56	16	40	0	0	0	190	54	136	1308	374	934
	Mar	18	5	13	240	66	174	4	1	3	144	40	104	2401	663	1738
	Apr	7	2	5	397	99	298	0	0	0	76	19	57	3611	901	2710
	May	56	2	54	500	16	484	2	0	2	217	7	210	3425	107	3318
	Jun	15	4	11	657	163	494	8	2	6	274	68	206	3943	980	2963
	Jul	6	1	5	593	136	457	13	3	10	95	22	73	4012	922	3090
	Aug	41	1	40	614	16	598	3	0	3	481	13	468	4133	108	4025
	Sep	17	4	13	617	153	464	0	0	0	227	56	171	3861	956	2905
	Oct	39	14	25	373	130	243	6	2	4	174	61	113	3009	1052	1957
	Nov	21	7	14	247	77	170	0	0	0	94	29	65	2406	749	1657
	Dec	11	3	8	113	32	81	0	0	0	169	48	121	917	259	658
Statistics	Mean	20.17			373.83			3			180.25			2791.17		
	SD	±16.59			±223.9			±4.16			±118.69			±1289.6		
	Range	2–56			56–657			0–13			22–481			468–4133		

*T = Total, M = Male, F = Female.

Table 3B
Month wise collection of *P. papatasi* from different habitats in Kala azar endemic areas of Malda, West Bengal.

Collection methods		Light trap collection									Aspirator collection					
Habitat		Human dwelling			Cattle shed			Peri-domestic vegetation			Human dwelling			Cattle shed		
		T	M	F	T	M	F	T	M	F	T	M	F	T	M	F
Months of collection	Jan	0	0	0	28	5	23	0	0	0	0	0	0	184	35	149
	Feb	0	0	0	18	5	13	1	0	1	13	4	9	379	108	271
	Mar	3	1	2	68	19	49	0	0	0	122	34	88	603	167	436
	Apr	12	3	9	112	28	84	9	2	7	83	21	62	1246	311	935
	May	3	0	3	206	6	200	3	0	3	204	6	198	996	31	965
	Jun	13	3	10	156	39	117	0	0	0	399	99	300	1106	275	831
	Jul	9	2	7	187	43	144	6	1	5	424	97	327	779	179	600
	Aug	19	0	19	112	3	109	11	0	11	189	5	184	1244	33	1211
	Sep	26	6	20	93	23	70	2	0	2	33	8	25	669	166	503
	Oct	2	1	1	136	48	88	2	1	1	67	23	44	556	194	362
	Nov	0	0	0	63	20	43	0	0	0	23	7	16	489	152	337
	Dec	6	2	4	26	7	19	0	0	0	71	20	51	232	65	167
Statistics	Mean	7.75			100.42			2.83			135.67			706.92		
	SD	±8.36			±62.78			±3.81			±144.14			±371.15		
	Range	0–26			18–206			0–11			0–424			184–1246		

*T = Total, M = Male, F = Female.

ing demonstrated that morphological identification was done correctly.

The majority of the sand flies were collected by aspirator from, cattle sheds followed by human dwellings. It was observed that sand flies were resting over the entire walls and on the ceilings. Sand flies were collected throughout the year from more than 70% of the houses and 50% of the rooms searched. The month-wise distribution of house positivity and room positivity are given in Fig. 3.

Only 6096 sand flies were collected by CDC light traps and the average no. of sand flies collected per light trap per night was 0.6. The number of sand flies collected by light traps from three different habitats significantly different in capture rates (Kruskal–Wallis Test, p - value <0.0001). By using Kruskal–Wallis pair-wise comparison test it was found that the sand flies trapped from cattle sheds were significantly higher than human dwellings ($p = 0.022$) and peri-domestic vegetation ($p < 0.0001$). But no such difference was recorded between human dwellings and peri-domestic vegetation ($p = 0.293$).

3.3. Impact of environmental factors on sand fly populations

The population density of sand flies was low during winter months from January to February and gradually reached the peak during summer and rainy seasons (Fig.-1). The highest man hour density was recorded from April to September (Fig.-4Figure 4:). Per night/ trap collection was also higher during the months of April to October (Fig.-5Figure 5:).

This indicates that the sand fly density increases with increasing average humidity, temperature, and rainfall. The Spear-

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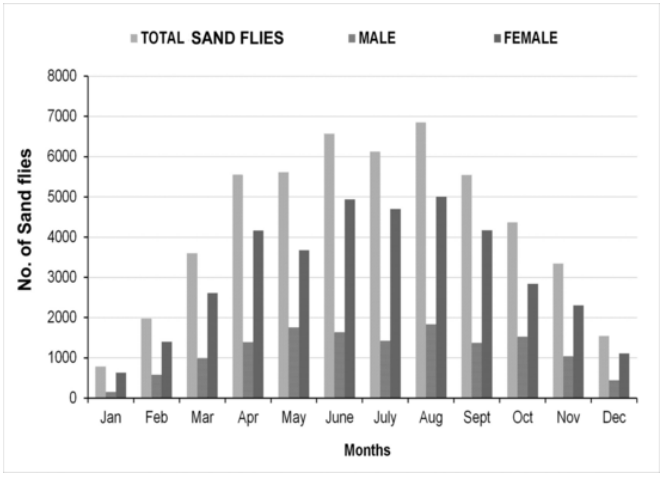

Fig. 1. . Distribution of sand fly by sex collected from Kala-azar endemic areas of Malda district, West Bengal.

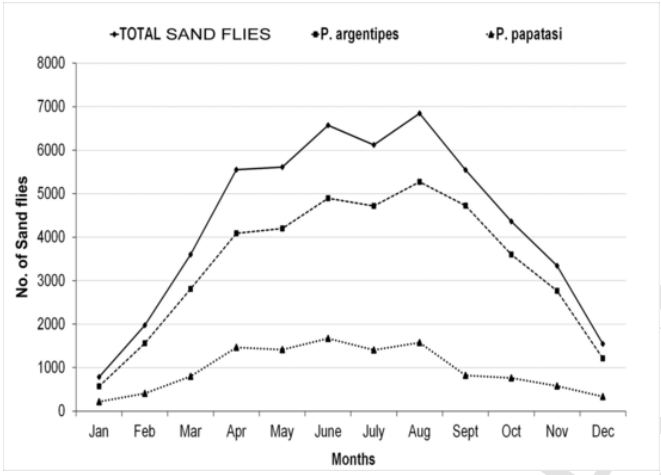

Fig. 2. Monthly dynamics of *P. argentipes* and *P. papatasi* collected from Kala- azar endemic areas of Malda district, West Bengal.

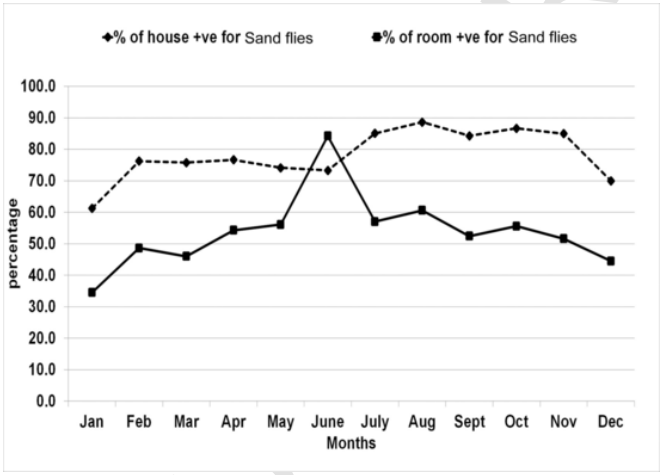

Fig. 3. Monthly variation of house positivity rate and room collection rates for sand flies in Kala-azar endemic areas of Malda district, West Bengal.

man's correlation analysis showed that the average humidity was positively correlated with sand fly density ($r = +0.782$, $p = 0.008$). A strong positive correlation was found between sand fly density and temperature ($r = 0.928$, $p = < 0.0001$), average rain

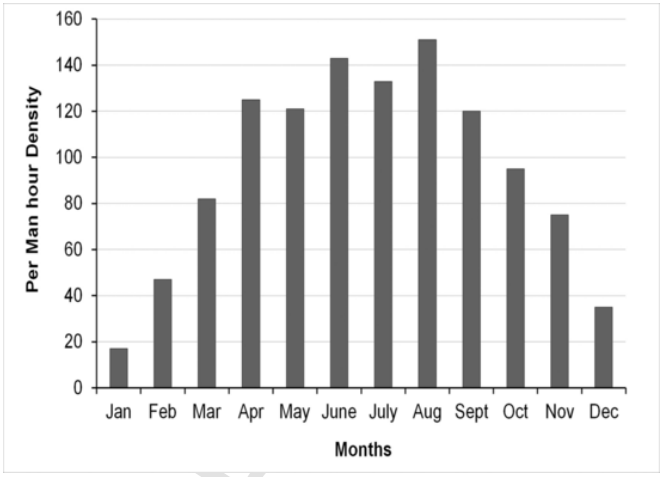

Fig. 4. Monthly Per Man hour Density (PMD) of sand flies in Kala-azar endemic areas of Malda district, West Bengal.

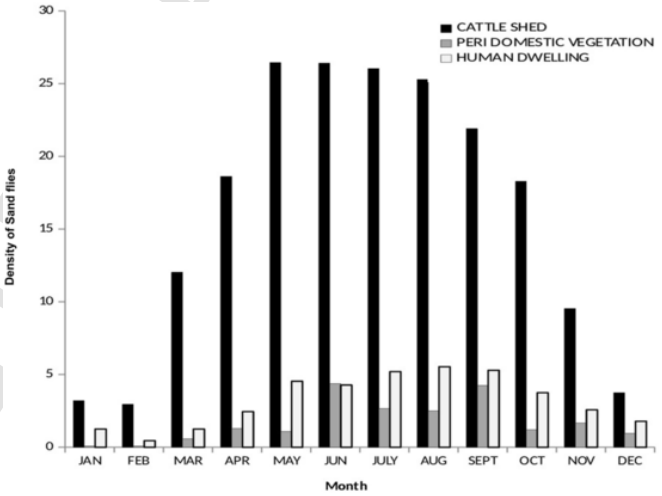

Fig. 5. Trap per night collection of sand flyies by habitat from Kala-azar endemic areas of Malda district, West Bengal.

fall ($r = 0.909$, $p = < 0.0001$). It is evident from the above analysis that temperature was most important environmental factors correlated with sand fly density.

4. Discussion

Long term vector borne disease control strategies require extensive studies on the ecological and behavioural aspects of vectors and their population dynamics pattern, changing with environmental factors. Climatic factors such as temperature, rainfall and humidity influence various aspects of vectors life cycles including larval development, vector pathogen interactions, and vector distribution. The vector of VL is a holo-metabolic insect completes its life cycle through egg, larva, pupa, and adult. Unlike the mosquito, there are no aquatic phases as larval and pupal stage requires a moist environment. Therefore, humidity and temperature affect on the sand fly life cycle.

In the present study, we were able to identify two different species of sand flies where *P. argentipes* was the predominant one. Various reports from different parts of India showed *P. argentipes* as predominant species (Srinivasan et al., 2013, 2014; Poche et al., 2018) along with other non-vector sand fly species. All collected sand flies were not analysed by molecular speciation methods which a limitation of this study.

The current vector control program for sand flies is primarily based on IRS with pyrethroid insecticides, considering endophagous and endophilic nature of sand fly behaviour (Gidwani et al., 2011). The exophilic and exophagous nature of *P. argentipes* has been documented (Poche et al., 2011). A significant proportion of outdoor *P. argentipes* population in peri-domestic vegetation was recorded from different villages of Bihar (Poche et al., 2011, 2012) and also from a higher canopy (Poche et al., 2017). This study we also collected *P. argentipes* from peri-domestic vegetation, but did not attempt to analyse the blood fed sand flies, therefore it is not to say about the source of the vertebrate blood for the outdoor dwelling sand flies. Previous studies showed feeding of human blood among sand flies collected from outdoor sources. In this study, villagers slept outside the house, particularly in summer, and might be the source of blood (WHO, 2012; Poche et al., 2018). As IRS has restricted use within the household and cattle sheds, therefore, a significant proportion of outdoor feeding sand flies did not come in contact with insecticide treated walls. The role of exophagic and exophilic *P. argentipes* in disease transmission is still unexplained. Policy makers should consider outdoor dwelling *P. argentipes* population for formulating a vector control strategy.

Cattle sheds are the most preferred breeding site for sand flies and provides favourable ecological conditions for reproduction. Greater abundance of *P. argentipes* in cattle sheds has been reported previously (Sanyal et al., 1979; Kumar et al., 1995; Dinesh et al., 2001). In this study we also found that abundance of sand flies was significantly higher in cattle sheds than in human dwellings.

Resting behaviour of sand flies was found to be restricted within 2 m from the ground level inside the human dwellings and cattle sheds (Hati et al., 1991). Thus, IRS of insecticide was restricted up to 2 m height of the walls in VL endemic areas. In our present study, it was observed that resting site of sand fly was not restricted to the lower portion of the walls. Sand flies were found at any heights and also on the ceilings. Such resting behaviour was also reported by other studies (Srinivasan et al., 2015). The sand fly population of the present study areas was found to be sensitive to pyrethroids (Sardar et al., 2018).

There are several methods for sampling for monitoring sand fly density such as - sticky trap, light trap, funnel trap, hand held mouth aspirator, and human landing (Alten et al., 2015). Each method has advantages and disadvantages that influence the actual density of sand fly (WHO, 1984). It was considered that human landing, sticky trap, and light trap collections were the standard methods for surveillance of sand fly populations (Killick-Kendrick., 1987). From an ethical stand point the human landing method for vector collection has been discarded due to risk of infection among volunteers (Das and Ramaiah, 2002). Although the sticky and light traps were recommended for monitoring sand fly population (Hoel et al., 2007; Orshen et al., 2010), but they has some limitations, as they can attract the sand flies only from a small distance from their breeding sites (Alexzander, 2000). In the present study we observed a significant difference between light trap collection and mouth aspirator collection. The light trap collection was comparatively lower than the aspirator collection that agrees with hypothesis of Burkett et al., 2007 but differs from the observations made by Poche et al., 2018, they collected more than 3000 sand flies per trap per night.

The distribution of sand flies and their population densities are largely dependent on environmental factors such as rainfall, temperature, and humidity (Sreenivisan et al., 2013; Poche et al., 2018). In the present study, we observed a linear relationship of sand fly density with temperature, humidity and rainfall. The peak density was recorded during April to September, i.e., during summer and monsoon. Similar observation was also noted from other regions of Indian sub-continent (Poche et al., 2011, 2018; Sreenivasan et al., 2013).

Temperature was found to be the most important predictor for population density rather than humidity and rainfall, as reported in our present study (Sreenivisan et al., 2013; Poche et al., 2018). Sand flies mainly breed in cattle sheds which maintains a high humidity throughout the year due to excreta from cattle. As there is no aquatic stage in the sand fly life cycle, therefore rainfall has no direct influence but it helps to maintain the humidity not only in breeding site but also in the local environment. This may be the cause behind the high population density in summer (Month of April, May, June) without sufficient rainfall. As insects are poikilothermic, a change in temperature in environment influences different developmental stages. Increases in temperature shorten the duration of developmental stages and increase the activity of insects (Tabachnick, 2010). While high temperature in summer might be the cause resulting in high population density of sand flies.

5. Conclusion

The present study villages of Malda district of West Bengal, showed *P. argentipes*, the VL sand fly vector was the predominant and most prevalent throughout the year. Sand flies are not only restricted to indoors, i.e., human dwellings and cattle sheds but also distributed outdoors in peri-domestic vegetation. A proportion of *P. argentipes* is both exophagic and exophilic. Cattle sheds were found to be the preferred habitat for *P. argentipes*. Therefore, cattle sheds should be sprayed properly with insecticides to reduce the vector density by treating resting sites. Sand flies were observed on walls of any height along with ceilings. The present method of use of insecticides on the walls is a best decision at this point and policy makers should consider about the outdoor sand fly population for disease control purposes.

Ambient temperature was the most important predictor for sand fly population density. Insecticides should be sprayed during early summer when the density is very low and second time after three months for a continued reduction of the sand fly population density.

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CRediT authorship contribution statement

Ashif Ali Sardar: Supervision, Investigation, Formal analysis, Writing - original draft, Writing - review & editing. **Moytrey Chatterjee:** Conceptualization, Supervision, Investigation, Formal analysis, Writing - original draft, Writing - review & editing. **Kingsuk Jana:** Formal analysis, Writing - original draft, Writing - review & editing. **Pabitra Saha:** Supervision, Investigation, Formal analysis, Writing - original draft, Writing - review & editing. **Ardhendu Kumar Maji:** Conceptualization, Supervision, Investigation, Writing - review & editing. **Subhasish Kamal Guha:** Conceptualization, Supervision, Writing - original draft, Writing - review & editing. **Pratip Kumar Kundu:** Supervision, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

We have no conflicts of interest concerning the work reported in this article.

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Supplementary materials

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