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A three year study on distribution and ecology of Anophelines in Thenzawl, Mizoram, India

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Abstract

A systematic survey on Anopheline species abundance, bionomics and habitat preference was conducted for three years in Thenzawl, Mizoram. A scoop–net method was employed for larval collection and a local made killing-jar for adults. A total of 10 species *Anopheles campestris* (25.8%), *An. nivipes* (24.0%), *An. vagus* (20.6%), *An. jamesii* (15.1%), *An. jeyporiensis* (11.4%), *An. maculatus* (1.7%), *An. philippinensis* (0.7%), *An. annularis* (0.26%), *An. sinensis* (0.23%) and *An. peditaeniatus* (0.22%) were collected. The survey site having thick tall grasses, numerous rural-huts as residents, small to relatively larger ponds and very slow running water bodies well shaded from sunlight with floating aquatic plants provided the largest area for *Anopheles* larvae breeding and accounted for 40% of all *Anopheles* larva and 25.4% total *Anopheles* spp. collected. *An. campestris* (NSK01), *maculatus* (NSK04), *philippinensis* (NSK06), *nivipes* (NSK10) and *jeyporiensis* (NSK09) were strongly anthropogenic and endophagic while *vagus* (NSK18) and *jamesii* (NSK03) were found to be highly zoophilic and exophilic and *An. peditaeniatus* (NSK02), *annularis* (NSK07) and *sinensis* (NSK15) were found to be highly zoophilic. Because of its abundance and bionomics, *An. campestris*, *jeyporiensis* and *nivipes* may have played a role in malarial transmission throughout the year. This is the first study reported on Anopheline distribution and abundance in Thenzawl, Mizoram.

Key words

Abundance, *Anopheles* species, Habitat preference, Malaria, Seasonal variation

Introduction

The *Anopheles* species is rich within the Oriental Region and occupies a wide variety of ecological niches (Foley *et al.*, 2007). Very few information on Anopheline prevalence, geographical distribution and relative density of potential vector populations has been reported from Mizoram. Anopheline survey in Mizoram indicated the incrimination of *Anopheles minimus* and *An. dirus* as malarial vectors (Prakash *et al*., 2006). The survey carried out by Rita *et al*. (2009; 2013) reported the presence of *An. philippinensis, An. subpictus, An. jamesii, An. varuna, An. jeyporiensis, An. vagus, An. maculatus, An. annularis, An. nivipes* and *An. minimus* in Mizoram. Further, other Anopheline species have also been reported as incriminated vectors in Northeast India (Bhattacharyya *et al*., 2010; Sarma *et al*., 2012a,b).

The relative humidity, temperature and rainfall of the region was reported to have a strong effect on the breeding and survival of mosquito vector and extrinsic incubation period of malaria parasite; the *r* – value (correlation coefficient) for temperature vs. *P. falciparum* and rainfall vs. *P. falciparum* were found to be 0.55 and 0.76, respectively indicating strong relationship between climatic factors and malaria (Pahwa and Dhiman, 2011). Predicted transmission window of malaria by 2030 showed stability since the climatic characteristics make the region highly conducive for mosquito breeding, survival and transmission (Dhiman *et al*., 2011).

War against malaria had started since 1957 in Mizoram and the State Vector-Borne Diseases Control Programme (SVBDCP), funded by the World Bank, had been setting up Full Therapeutic Dose (FTD) in all towns and villages to help malaria patients get treatment on time. For vector control, the entire state had been targeted for two rounds of 1% DDT spray per annum (IRS) and distribution of 1% K-othrine® (2.5% delthamethrin w/w) treated bed-nets (ITN) in rural areas (Anonymous, 2010).

Malaria is prevalent throughout the year in Thenzawl, and majority (99%) of the population suffered with malaria. However, mortality rate was quite low (0.15%), during the three year study, perhaps because of timely treatment (Anonymous, 2011). Further, implementation of the Anti-malaria Programme by SVBDCP since April 2010, reduced the malarial incidence to 74.77% (2009), 22.02% (2010) and to 3.21% (2011) (Anonymous, 2011).

The undertakings of the SVBDCP, Mizoram towards malarial eradication had solely focused only on the human treatment, IRS and ITNs. An effective and efficient surveillance system should also prioritize on the entomological component viz. *Anopheles* habitat and seasonal abundance, adult populations and the transmission of the vector - borne disease. In the present study, identification, habitat and abundance of *Anopheles species* within Thenzawl were carried out in relation to malarial prevalence.

Materials and Methods

Site, sampling and identification : Thenzawl is characterized by numerous slow-running low streams, concrete and earthen irrigational canals and drainage and ditches; and artificial ponds (2–9 m in diameter) which was used for harvesting fishes. *Anopheles* species habitats were surveyed within 2-4 km radius of the town (S1- human inhabitation with stream and water storage tanks; S2- forest reserve having ponds and natural vegetation; S3- Rural area with rock holes and natural vegetation; S4- Grassland ecosystem with pond; S5- Urban area with water canals; S6- Market area with piggery stands and water canals; S7- hospital area with drainage; S8- Jhum cultivation areas; S9- Fast flowing stream with natural vegetation; S10- Urban area with water storage tanks) from January to November for three years (2009 -2011) (Table 1). The larval habitats were characterized by clear water (5.0 – 6.2 pH; 22 – 30°C temperature) interrupted with floating aquatic plants and dead/fallen plant debris and were shaded by a vegetation of trees and tall grasses. Adults were collected at dusk and midnight (16:30 – 20:00 hrs; 12:00 – 4:00 hrs) from cattle sheds, nearby human residents and agricultural lands (23 – 36°C; 20 – 98% RH).

Larvae were collected by the scoop-net method, with larval net of a fine mesh net (10 x 10 finely knitted threads per cm^2) mounted to an iron handle (30 cm diameter), plastic tub (28.5 cm diameter), a plastic dipper (15.5 cm diameter) and a dropper (Oo *et al.*, 2004). Adults were collected using a CDC (Centers for Disease Control and Prevention) –light trap and local-made killing jar which consisted of a 250 ml glass jar (2 cm in diameter, 9.7 in length) and cotton moistened with chloroform, kept at the base of the jar. Each paralyzed/dead adult was immediately transferred to sterile 1.5 ml micro-centrifuge tube (Tarson) that contained silica-gel and cotton. Morphological identification of adult female was done based on the keys of palpi, wing and legs as described by Das *et al.* (1990) and Nagpal and Sharma (1987; 1995).

Statistical analysis : Statistical calculations were performed using commercially available GraphPad InStat version 3.06 software (GraphPad Software Inc., San Diego, CA) and PAST 1.86b (Hammer *et al.*, 2001). One way ANOVA was used to compare species abundance and malarial prevalence. Levene's test (based on means) was calculated to find the homogeneity of variances within *Anopheles* population. A *p*-value of < 0.05 was noted to have statistically significant value.

Results and Discussion

The present study revealed that the abundance of *Anopheles* species were slightly low in 2011 (32.4% of total *Anopheles* species) as compared to 2009 (34.2%) (Table 1; Fig. 1). There were no significant statistical differences in the total number of *Anopheles* collected in 2009 ($F_{235.3} = 7.5$; P = 2.717E-08), 2010 ($F_{315.9} = 7.3$; P = 3.943E-08), and 2011 ($F_{326.9} = 7.5$ P = 2.853E-08). Levene's test (based on means) gave significant homogeneity of variances within the *Anopheles* populations $W_{(2009)}$ = 1.167E-15, $W_{(2010)}$ = 1.549E-13 and $W_{(2011)}$ = 1.38E-14. In the present survey, the most dominant species was *An. campestris* (NSK01) (25.8%) followed by *An. nivipes* (NSK10) (24.0%.), *An. vagus* (NSK18) (20.6%), *An. jamesii* (NSK03) (15.1%), *An. jeyporiensis* (NSK09) (11.4%), *An. maculatus* (NSK04) (1.7%), *An. philippinensis* (NSK06) (0.7%), *An. annularis* (NSK07) (0.26%), *An. sinensis* (NSK15) (0.23%) and *An. peditaeniatus* (NSK02) (0.22%), respectively (Table 2).

The monthly proportion of total *Anopheles* collected gradually increased from February (2.8%), attained its maximum by May – June (21 – 23.1%) and gradually decreased by November (0.85%) (Table 2). The most consistent species throughout the year were *An. campestris* (NSK01) (Jan – 0.5%; Nov – 2.6%) and *An. vagus* (NSK18) (Jan – 0.8; Nov – 0.9%), *An. campestris* reached its maximum in June (20.6%) while the latter in May (27.7%). *An. jamesii* (NSK03) and *An. nivipes* began its prevalence in February (0.89% and 3.80%, respectively), rose to maximum during monsoon period (May - 34.0% and 22.2%, respectively) and receded in post-monsoon period (October - 1.6% and 0.5%, respectively). Similar pattern was observed by Malhotra (1994).

Maximum abundance of Anopheline species was observed during pre-monsoon and post-moonsoon season (Aung *et al.*, 1999), while for some species during monsoon season (Bansal and Singh, 1993). Amerasinghe *et al.* (1999) reported that rainfall affect Anopheline abundance significantly.

Very slow running water of temperature 24 - 27°C, pH 5.2 - 5.5, well shaded from sunlight, with aquatic vegetation coverage (riparian, floating, and emergent) provided the largest area for *Anopheles* larvae breeding and accounted for 40% of all

Table 1 : Global positioning system, temperature and relative humidity of survey area with its Anopheles species distribution at Thenzawl, January – November, 2009 – 2011. + indicates presence and – indicates absence

Month	No. of sites surveyed	Spp. present / site (%)	Total spp. collected (%)	Mean of species collected	No of sps. for test	NSK01	NSK02	NSK03	NSK04	NSK06	NSK07	NSK10	NSK15	NSK 09	NSK18
Jan	10 (100%)	3 (30 %)	77 (0.4%)	7.7 ± 4.0	10 (13.0%)	27 (0.14%)	-	-	-	-	-	-	-	19 (0.1%)	31 (0.16%)
Feb	10 (100%)	9 (90 %)	536 (2.8%)	53.6 ± 22.8	10 (1.9%)	60 (0.31%)	4 (0.02%)	26 (0.13%)	10 (0.05%)	-	2 (0.01%)	177 (0.92%)	2 (0.01%)	187 (0.97%)	68 (0.35%)
Mar	10 (100%)	10 (100 %)	1472 (7.6%)	147.2 ± 50.2	10 (0.7%)	283 (1.47%)	3 (0.02%)	146 (0.76%)	24 (0.12%)	4 (0.02%)	4 (0.02%)	380 (1.97%)	3 (0.02%)	286 (1.48%)	339 (1.76%)
Apr	10 (100%)	10 (100 %)	2413 (12.5%)	241.3 ± 86.2	10 (0.4%)	483 (2.5%)	6 (0.03%)	307 (1.59%)	36 (0.19%)	10 (0.05%)	6 (0.03%)	759 (3.93%)	5 (0.03%)	287 (1.49%)	514 (2.66%)
May	10 (100%)	10 (100 %)	4451 (23.1%)	445.1 ± 157.5	10 (0.2%)	1011 (5.24%)	7 (0.04%)	989 (5.13%)	44 (0.23%)	18 (0.09%)	7 (0.04%)	1034 (5.36%)	7 (0.04%)	312 (1.62%)	1022 (5.30%)
Jun	10 (100%)	10 (100 %)	4068 (21.1%)	406.8 ± 143.5	10 (0.2%)	1025 (5.31%)	7 (0.04%)	671 (3.48%)	54 (0.28%)	33 (0.17%)	9 (0.05%)	1028 (5.33%)	8 (0.04%)	311 (1.61%)	922 (4.78%)
Jul	10 (100%)	10 (100 %)	2697 (14.0%)	269.7 ± 93.2	10 (0.4%)	771 (4.0%)	3 (0.02%)	352 (1.82%)	59 (0.3%)	35 (0.18%)	9 (0.05%)	697 (3.61%)	8 (0.04%)	315 (1.63%)	448 (2.32%)
Aug	10 (100%)	10 (100 %)	1867 (9.68%)	186.7 ± 64.2	10 (0.5%)	614 (3.18%)	7 (0.04%)	233 (1.21%)	65 (0.34%)	19 (0.1%)	6 (0.03%)	357 (1.85%)	7 (0.04%)	276 (1.43%)	283 (1.47%)
Sep	10 (100%)	10 (100 %)	1089 (5.6%)	108.9 ± 36.4	10 (0.9%)	335 (1.74%)	6 (0.03%)	139 (0.72%)	26 (0.13%)	13 (0.07%)	6 (0.03%)	203 (1.05%)	3 (0.02%)	170 (0.88%)	188 (0.97%)
Oct	10 (100%)	8 (80 %)	463 (2.4%)	46.3 ± 23.9	10 (2.2%)	236 (1.22%)	0 (0.0	47 (0.24%)	4 (0.02%)	5 (0.03%)	2 (0.01%)	21 (0.11%)	1 (0.01%)	31 (0.16%)	116 (0.60%)
Nov	10 (100%)	2 (20 %)	164 (0.9%)	16.4 ± 12.8	10 (6.1%)	127 (0.66%)	- -	- -	- -	- -	- -	- -	- -	- -	37 (0.19%)
	110	10	19297		110	4972 (25.8%)	43 (0.22%)	2910 (15.1%)	322 (1.7%)	137 (0.7%)	51 (0.26%)	4656 (24.0%)	44 (0.23%)	2194 (11.4%)	3968 (20.6%)

Anopheles species collected. Such habitat environment that favored larval density was also observed by Devi and Jauhari (2008); Minakawa *et al.* (2005); Stoops *et al*., (2007) and Mala *et al.* (2011). Adults were mainly collected from areas with ranging from 22 - 36°C and 20 – 98.0% relative humidity throughout the year. *An. campestris* (NSK01) (p>0.1), *An. maculatus* (NSK04) (p>0.1), *An. philippinensis* (NSK06) (p>0.1), *An. nivipes* (NSK10) (p>0.1) and *An. jeyporiensis* (NSK09) (p>0.1) were strongly anthropogenic and endophagic and could be collected more often indoors than outdoors, while *An. vagus* (NSK18) (p>0.1) and *An. jamesii* (NSK03) (p>0.1) were found to be highly zoophilic and exophilic and were mainly collected from outdoors. *An. peditaeniatus* (p>0.1), interestingly for *An. annularis* (NSK07) (p>0.1) and *An. sinensis* (NSK15) (p>0.01) were found to be highly zoophilic as they could be found only in indoor cattle–sheds.

Maximum collection of *Anopheles* species was observed in permanent ponds (site – 3; 25.4%) with vegetation followed by temporary ponds (site – 2; 10%), and least in shallow pits and seepages (site 8; 4.8%) (Table 2). Hence, distribution of *Anopheles* species was found to be non-random and predictable on the basis of habitat characteristics, thereby confirming the observations made by Stoops *et al*. (2007); Devi and Jauhari (2008) and Kenea *et al*. (2011).

Malaria was predominant throughout the year in Thenzawl. From the total monthly collections of *Anopheles* species from 2009 to 2011, *An. campestris* (NSK01), *An. nivipes* (NSK10) and *An. jeyporiensis* (NSK09) were found most dominant not only in all survey sites but also within the residential area of the malaria patients. During winter months (January and February), the population of *An. campestris* (NSK01) (0.54 – 1.21%) and *An. jeyporiensis* (NSK09) (0.87 – 8.52%) exhibited high abundance along with malarial prevalence (1.83 – 2.29%) of the total reported malaria cases (Fig. 2) (NRHM HMIS report, 2009; NVBDCP, 2010; Mizoram Health and Family Welfare Department, 2011). During the summer months (March, April and May), *An. campestris* (5.69, 9.7, and 20.3%, respectively), *An. nivipes* (8.16, 16.3 and 22.2%, respectively) and *An. jeyporiensis* (13.04, 13.08 and 14.22%, respectively) were high in population along with a slight increase in the incidence of malaria (1.38, 8.26 and 15.6%, respectively) (Figure 2). *An. campestris* (NSK01) surged to its maximum abundance of 20.62% during the commencement of monsoon month (June) with corresponding increase in the number of malaria cases (16.1%). Monsoon further prevailed during July and August and the number of *An. campestris* (15.5 and 12.35%), *An. nivipes* (NSK10) (14.97 and 7.67%) and *An. jeyporiensis* (NSK09) (14.36 and 12.58%) populations were high in correspondence with the malaria cases reported (26.1 and 15.1 %), respectively. October and November witnessed the receding of monsoon and so were *An. nivipes* (0.45%) and *An. jeyporiensis* (1.41%) in October and disappeared by November. Meanwhile *An. campestris* prevailed with 4.75 and 2.55% by October and November together with malaria cases (3.67 and 1.83%).

Rise or decrease of temperature and rainfall can affect the density of Anopheline population (van den Hurk *et al*., 2000; Gilioli and Mariani, 2011; Imbahale *et al*., 2011). The three year study showed slight increase in temperature and annual rainfall with maximum of 25.3°C and 248.09 cm (2009) to 26.3°C and 330.45 cm (2011), respectively which favored slight increase in *An. maculatus* (NSK04) and *An. philippinensis* (NSK06) while *An. annularis* (NSK07), *An. nivipes* (NSK10) and *An. vagus* (NSK18) decreased. The environmental factors that influenced the proportion and abundance of *Anopheles* species as observed in the present study were the onset of monsoon rains (highly variable annually) and occasional cyclone (Kim *et al.,* 2011); agricultural practices (Minakawa *et al.*, 2005; Jacob *et al*., 2007;

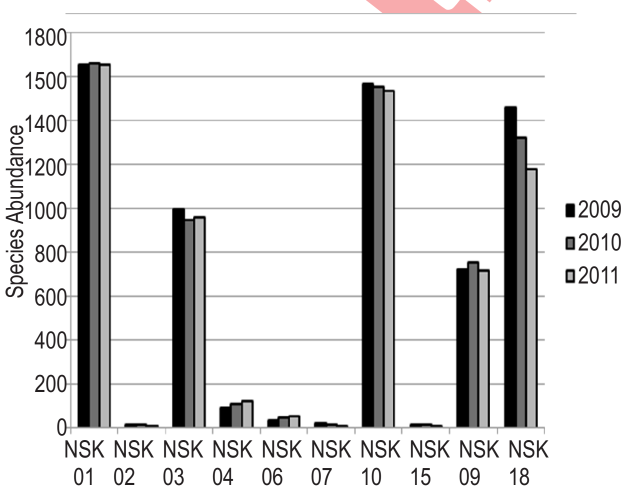

Fig. 1 : Yearly percent of *Anopheles* spp. collected Yearly (A) at Thenzawl, January – November, 2009 – 2011

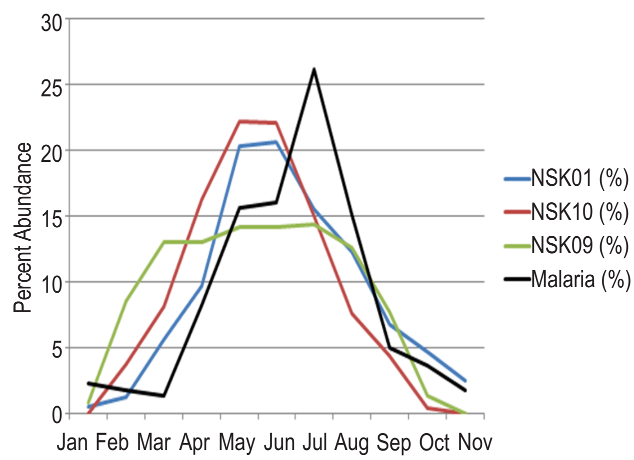

Fig. 2 : Relation of *Anopheles* spp. abundance with malaria prevalence at Thenzawl, January – November, 2009 – 2011

Table 2 : Global positioning system, temperature and relative humidity of survey area with its *Anopheles* species distribution at Thenzawl, January – November, 2009 – 2011. + indicates presence and–indicates absence.

Survey Site	Coordinates	Elevation	Temperature / RH	*An.* (A) *campestris* NSK 01	*An.* (A) *peditaeniatus* NSK 02	*An.* (C) *jamesii* NSK 03	*An.* (C) *maculatus* NSK04	*An.*(C) *philipinensis* NSK 06	*An.* (C) *annularis* NSK 07	*An.*(C) *nivipes* NSK 10	*An.* (A) *sinensis* NSK 15	*An.* (C) *jeyporiensis* NSK09	*An.* (C) *vagus* NSK 18
S1	23°17'13.21"N 92°46'50.58"E	2449ft	23 - 34°C / 20-80%	+	-	+	+	-	-	+	-	+	+
S2	23°17'12.27" 92°46'55.65"E	2506ft	24 - 33°C / 28-80%	+	-	+	-	-	-	+	-	-	+
S3	23°17'10.01"N 92°46'49.35"E	2457ft	25 - 36°C / 30-98%	+	-	+	+	-	-	+	-	+	+
S4	23°16'51.98"N 92°46'49.64"E	2505ft	26 - 32°C / 34-79%	+	-	-	-	+	-	+	-	-	+
S5	23°16'47.49"N 92°46'29.01"E	2539ft	27 - 32°C / 30-90%	+	-	-	-	+	-	+	-	-	+
S6	23°17'1.02"N 92°46'28.86"E	2517ft	22 - 34°C / 20-80%	+	-	+	+	-	-	+	-	+	+
S7	23°16'28.19"N 92°46'19.20"E	2464ft	29 - 32°C / 50-80%	+	-	+	-	-	-	+	-	+	+
S8	23°17'26.77"N 92°46'19.88"E	2726ft	30 - 32°C / 50-80%	+	+	-	-	-	-	+	-	-	+
S9	23°16'53.93"N 92°46'4.42"E	2603ft	24 - 33°C / 28-80%	+	+	+	+	+	-	+	+	+	+
S10	23°17'10.33"N 92°46'15.40"E	2563ft	25 - 36°C / 30-98%	+	+	+	+	+	+	+	+	+	+

Table 3: *Anopheles* collected (in numbers) at different survey sites at Thenzawl during Jan – Nov. 2009 – 2011

Site	NSK01	NSK02	NSK03	NSK04	NSK06	NSK07	NSK10	NSK15	NSK09	NSK18	Total%
S1	335	-	281	72	-	-	403	-	375	216	8.7
S2	767	-	492	-	-	-	443	-	-	229	10
S3	2228	-	959	42	-	-	1102	-	360	211	25.4
S4	367	-	236	-	29	-	348	-	-	332	6.8
S5	265	-	0	-	37	-	399	-	-	1118	9.4
S6	160	-	234	65	-	-	350	-	375	435	8.4
S7	154	-	215	-	-	-	415	-	367	339	7.7
S8	166	12	-	-	-	-	379	-	-	368	4.8
S9	256	17	254	69	29	-	424	19	354	345	9.2
S10	274	14	239	74	42	51	393	25	363	375	9.6
Total spp:	**4972**	**43**	**2910**	**322**	**137**	**51**	**4656**	**44**	**2194**	**3968**	**100**

Mwangangi *et al*., 2010) including use of fertilizers (Darriet *et al*., 2012) and inter or intra-specific competition (Schneider *et al*., 2000; Knight *et al*., 2004).

A total of 218 malaria cases were reported in Community Health Centre, Thenzawl, within 2009 – 2011 and *P. falciparum* was more prevalent (80.7%) than *P. vivax* (18.3%), with few mixed infections (0.92%). The monthly abundance and bionomics of *An. campestris* (NSK01), *An. maculatus* (NSK04), *An. jeyporiensis* (NSK09) and *An. nivipes* (NSK10) species in Thenzawl may have implications to malarial cases and be responsible for malarial transmission in Thenzawl. It was also noted that some *Anopheles* species have tendency to rest in animal shelters after feeding on human (Basseri *et al*., 2010), as some species described (such as *An. annularis* and *An. sinensis*) in the study, did not show their role in malaria transmission because of their zoophilic behavior (Manguin *et al*., 2008). The vector potential of *An. campestris* (Limrat *et al*., 2001) and *An. maculatus* (Rongnoparut *et al*., 1996) in Thailand; *An. nivipes* in North East India (Prakash *et al*., 2005) has been reported.

In the present study, monthly proportion of total adult *Anopheles* collected gradually increased from February (2.8%), attained its maximum by May - June (23.1%) and gradually decreased by November (0.85%). Incubation of the malaria parasite fluctuates with temperature and it generally takes 9 to 30 days to infect (Paaijmans *et al*., 2011), which relates to the *Anopheles* species abundance in June and surge of malaria during July in Thenzawl.

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