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Effect of environmental parameters on the relative abundance of immature stages of mosquitoes in Mizoram, India

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ABSTRACT

A study to assess mosquito species occurrences and the effects of some ecological characteristics on their breeding was undertaken in Mizoram during 2010-2013. A total of 8328 mosquitoes, representing the 5 genera of: *Anopheles*, *Aedes*, *Culiseta*, *Culex* and *Toxorhynchites* were collected altogether of 20 species. They were found in a wide variety of habitats with the altitudinal variation of 54 - 1150m. The zones of lower elevation shared higher species abundance than the higher elevation. The most dominant genus was *Aopheles* followed by *Culex*, *Culiseta*, *Aedes* and *Toxorhynchites.* The results of forward multiple regression analyzed the effect of environmental parameters on the relative abundance of immature stages of mosquitoes. These included dissolved oxygen, TDS, temperature, alkalinity, pH and hardness.

KEYWORDS

Water quality;
Mosquito;
Relative abundance;
Density.

INTRODUCTION

Mosquitoes (Diptera: Culicidae) and mosquito-borne diseases have been threatening human and animals. There are 38 genera of mosquitoes worldwide wherein three genera (*Anopheles*, *Aedes* and *Culex* were the most important one transmitting dengue fever, yellow fever, malaria, filariasis, chikungunya and encephalitis[1]. No part of the world is free from vector borne diseases. Mosquito-borne parasitic diseases are endemic in many areas of the world, causing more than 3.2 billion people to be at risk[2].

Understanding where mosquitoes breed and why they prefer certain water bodies over others is vital for designing mosquito control strategies. Knowing the ecology and behavior of a vector is essential to determine its role in disease transmission and the type of control measures that may be appropriate for it[3]. Several work being done through distribution and types of larval breeding habitats. Mosquitoes are more abundant in temporary breeding places while others occurred usually in permanent ones[4], The resources in terms of food, predators and competitors present in the habitat determine the population status of larval mosquitoes, both qualitatively and quantitatively[1]. A number of studies have been carried out on mosquito breeding in various habitats[5-8], one of the most important factors was the vegetation that favours larval propagation and is correlated with adult densities[9-10]. Small and open habitats are more productive and selected for oviposition sites compared to large larval habitats during the rainy seasons[11].

The distribution and abundance of an insect species depends on its own biological characteristics and the influence of other organisms, on its physical environment. Weather plays a major role[12], as insects are poikilothermic or cold-blooded. Metabolic heat, which is generated by most insects themselves, is limited and has little effect on their body temperature. Therefore, their metabolic rate and the growth and development rate of insects depend on the temperature of their direct environment. Temperature, rainfall and relative humidity are physical factors that influence the abundance of the mosquitoes[13].

Rainfall provides the breeding sites for mosquitoes and increases relative humidity necessary for mosquito survival, leading to increase in human biting rate. Humidity is one of the most important environmental factors affecting the mosquito distribution[14]. Almiron and Brewer, Rajnikant *et al.* and Reisen *et al.* demonstrated interspecific associations among mosquitoes and a correlation with physico-chemical and biological composition of mosquito breeding waters. Mosquitos' species differ in the type of aquatic habitats, they prefer for oviposition based on location, the physico-chemical condition of the water body, and the presence of potential predators[3,15]. *Anopheles* mosquito has been found to use fresh water habitats for breeding[16]. Larvae of *Anopheles* mosquitoes in clear water of suitable temperature and nutrient conditions have been found to thrive in aquatic bodies several studies have examined the relationship between habitat characteristics and larval abundance. In Sri Lanka, *An. culicifacies* was positively associated with light and vegetation and negatively associated with the presence of potential predators, while *An. varuna* was positively associated with a variety of aquatic fauna[15]. In Venezuela, salinity and dissolved oxygen were associated with the spatial distribution of *An. aquasalis* and *An. oswaldoi*. *Cx. quinquefasciatus* larvae, in Peninsular Malaysia, were most abundant in polluted drains containing 1.0 to 2.0 g/liter of dissolved oxygen, 1.0- 2.4 g/liter of soluble reactive phosphate, and 0.1-0.9 g/liter of ammonical nitrogen[17]. Studies of Kengluecha *et al*. in Thailand stated that, water hardness was probably responsible for the dominance of *An. minimus* while negative relationship between pH, *An. dirus* larvae was found in habitats with lower pH values especially in the ground pools. However, edges of streams and rivers leads *An.* species larval deaths due to reduction in small, temporary rain pools[16] as well as high water current and flooding have been reported to lead to *An.* species larval deaths due to reduction in oxygen tension causing physical harm to the larvae[18]. Physico-chemical factors that influence oviposition, survival, and the spatio-temporal distribution of important disease vector species include salts, dissolved organic and inorganic matter, turbidity, presence of suspended mud, presence or absence of plants, temperature, light and shade and hydrogen ion concentration[19]. *An. vagus* prefers a higher pH for rice paddy field whereas *An. campestris* prefer a higher concentration of dissolved oxygen in the swamp[18]. Therefore, the present study is the first attempt to understand mosquito associations in different breeding habitats and to characterize the physico-chemical conditions of these mosquito larval habitats, to identify the factors that influence the abundance and distribution of mosquito in diverse aquatic habitats in Mizoram, India.

MATERIALS AND METHODS

Study area

The study covered a major part of the six districts in Mizoram (between 2010-2013) including Aizawl (23°44' N, 92°42' E), Serchhip (23°16' N, 92°44' E), Mamit (23°55' N, 92°29' E), Lunglei (22°52' N, 92°43' E), Lawngtlai (22°18' N, 92°41' E) and Kolasib (23°13' N, 92°40' E) with the altitudinal variation of 54 - 1150 m. Priority on the sites of collection was mainly based on malarial prevalences and occurrences data obtained from Health Dept. Govt. of Mizoram. These were considered as probable mosquito larval habitats: (i) cemented pools (cemented walls), (ii) ponds, (iii) household water storage tanks (barrels), (iv) stagnant stream side pools, (v) temporary ditches, (vi) shallow pits and (vii) seapage pool, (viii) cattle sheds, (ix) human residents.

Larva collection (scoop-net method)

The water bodies (ponds, ditches, pools, river beds, tree holes, rock holes, tanks and containers) were surveyed and subsequently sampled, collection of immature mosquitoes was also made on the same day (8:00

Figure 1: Map of Mizoram showing different districts

am – 3:00 pm) by the scoop-net method[20], with a larval net of a fine mesh net mounted to a iron handle (25 cm diameter), plastic tub of different sizes, plastic dipper and dropper (21 - 38°C; 25 - 98% RH). Larvae collected in the field were sorted and segregated depending on Anopheline and Culicine larvae. It was then immediately carried to laboratory for further analysis.

Identification of mosquito

Morphological identification of mosquito was done on adult female taking colour pattern of wing, palpi and leg as identification characters using dissecting light microscope and hand lens. The identification keys followed the illustration of Das *et al*.[8], Glick[22], Reuben *et al*.[23], Nagpal and Sharma[24], Oo *et al*.[25].

Water quality measurement

Water samples were taken from the different positive breeding such as ponds, river beds, ditches and pools within Aizawl district during 2010-2013. It was then immediately carried to laboratory for analysis of different parameters and to find out the best predictor for larval abundances. The following parameters of water quality were measured: temperature, *p*H, dissolved oxygen (DO), Hardness. Alkanility, Phosphate, Chloride and Total dissolved solids. Samples for dissolved oxygen (DO) were collected into dark bottles. pH and temperature were taken *in situ*. Water turbidity was estimated visually against a white background and classified as either clear, less turbid, or turbid. On each sampling, 200 ml of water was collected from each habitat, using a standard dipper from the sites where the mosquito larvae were sampled. The sample was fixed in the field for estimating the amount of dissolved oxygen present in the water by the Winkler's method. The water quality analysis was done as per Trivedy *et al*.[26] and American Public Health Association[27].

TABLE 1 : Parameters and their methods of determination

Parameter	Method employed
pH	pH paper and Eutech instrument pc 510
Temperature	Thermometer (Mercury in glass thermometer)
Alkalinity	Strong acid titration method
Dissolved Oxygen	Azide modification of Winkler's method
Hardness	Complexometric titrations method
Phosphate	Stannous chloride reduction method
Chloride	Mohr's method
Total Dissolved Solids	Gravimetric method

Data analyses

Data were analyzed using GraphPad instat Version 3.0 (GraphPad software inc., San diego, CA) and PAST 1.86b statistical packages. The relative abundance of mosquitoes was expressed as the number of mosquito larvae and pupae per 20 dips because larval and pupal counts were low. The differences in larval counts among habitat types were compared by repeated measures analysis of variance (ANOVA). Forward multiple regression analysis was used to obtain the best predictor variables explaining the abundance of the mosquito larvae. Statistical analysis was done after a log transformation log10 (n+1) of larval abundance values to normalize the distribution and minimize the standard error (SE).

RESULTS

A total of 8328 mosquitoes, representing the 5 genera of: *Anolpheles*, *Aedes*, *Culiseta*, *Culex* and *Toxorhynchites* were collected altogether of 20 species (TABLE 2). They were found in a wide variety of habitats with the altitudinal variation of 54 - 1150m. The zones of lower elevation shared higher species abundance than the higher elevation. The most dominant genus was *Anolpheles* followed by *Culex*, *Culiseta*, *Aedes* and *Toxorhynchites*. In the overall survey, the

TABLE 2 : Distribution and abundance of collected mosquitoes across six districts in Mizoram 2009-2011

Collection sites	*Cxm*	*Cum*	*Txr*	*Cxt*	*Cxu*	*Cxq*	*Cxr*	*Cxb*	*Aea*	*And*	*Anb*	*Cui*	*Anv*	*Anj*	*Ani*	*Aey*	*Ann*	*Anm*	*Anp*	*Anw*
Aizawl District																				
Lengpui	213	-	-	-	-	856	245	156	140	-	1250	-	985	-	12	-	-	-	20	-
Dinthar	-	-	23	-	-	126	-	-	-	-	12	-	-	-	-	-	-	-	-	6
Sihhmui	18	-	-	-	-	248	8	6	56	-	185	-	53	-	-	-	-	-	-	-
Ramrikawn	-	-	-	85	68	128	-	-	5	-	-	-	6	-	-	-	-	-	-	-
Mission	-	14	5	-	-	-	-	-	-	-	-	-	13	-	-	-	-	-	8	-
sub total	231	14	28	85	68	1358	253	162	199		1447		1057		12				28	6
Kolasib District																				
Diakkawn	-	48	15	-	-	486	-	-	-	-	235	28	-	2	-	-	-	-	6	-
Rubber board	-	-	-	-	-	235	-	-	-	-	123	-	45	-	-	-	-	-	-	-
Rengtekawn	-	5	2	-	-	457	-	-	-	-	85	-	-	-	-	-	-	-	-	-
sub total		53	17			1178					443	28	45	2					6	
Serchhip District																				
Ponds	-	-	-	-	-	235	-	-	45	-	156	-	8	-	-	-	-	-	-	-
Rock holes	-	-	-	-	-	-	-	-	-	-	86	-	36	2	8	-	12	4	2	-
Rice field	-	-	-	-	-	225	-	-	-	-	12	-	6	-	-	-	-	-	-	-
sub total						460			45		254		50	2	8		12	4	2	
Mamit District																				
Lungsir	-	-	-	-	-	15	-	-	-	-	23	-	-	-	-	-	-	-	-	-
Bawngva	-	-	-	-	-	28	-	-	-	2	52	-	45	-	-	-	-	-	4	4
Darlak	-	-	-	-	-	45	-	-	5	-	-	-	-	-	4	2	-	2	-	2
sub total						88			5	2	75		45		4	2		2	4	6
Lunglei District																				
Lunglei park	-	-	9		-	18	-	-	23	-	12	-	-	-	-	-	-	-	-	-
Chawngte	-	-	-	-	-	135	-	-	-	1	82	-	6	5	-	-	4	-	-	-
sub total			9			153			23	1	94		6	5			4			
Lawngtlai District																				
Ponds	-	-	-	-	-	21	-	-	-	-	120	-	5	-	-	-	-	-	-	-
Tanks	-	-	-	-	-	45	-	-	28	-	12	-	-	-	-	-	-	-	-	-
sub total						66			28		132		5							
G.Total	231	67	54	85	68	3303	253	162	302	3	2455	28	1208	9	24	2	16	6	40	12

most dominant species was found to be *Cx. quinquefasciatus* (39.67%) followed by *An. barbirostris* (29.50%), *An. vagus* (14.51%), *Ae. albopictus* (3.62%), *Cx. tritaeniorhynchus* (3.03%), *Cx. mimeticus* (2.78%), *Cx. bitaeniorhyunchus* (1.94%), *Cx. tarsalis* (1.02%), *Cu. melanura* (0.80%), *Cx. peus* (0.82%), *Tx. splenden* (0.64%), *An. philipinensis* (0.48%), *Cu. inornata* (0.33%), *An. jamesi* (0.29%), *An. nivipes* (0.19%), *An. willmori* (0.14%), *An. jeyporiensis* (0.10%), *An. minimus* (0.07%), *An. dirus* (0.03%) and *Ae. aegypty* (0.02%).

The results of forward multiple regression analyzed the effect of environmental parameters on the relative abundance of immature stages of mosquitoes in Aizawl district. The model included 22 variables: 8 physico-chemical variables (TABLE 3) and 14 species of mosquitoes identified in the samples. Six of the 8 physico-chemical parameters parameters were significantly associated with the relative abundance of immature mosquitoes (TABLE 4); these included dissolved oxygen,

TABLE 3 : Average values (mean± SE) of the measured environmental factors (water quality) in Aizawl

Study site	pH (mg/l)	Alkalinity (mg/l)	Hardness (mg/l)	Temp. (C)	DO (mg/l)	TDS (mg/l)	Phosphate (mg/l)	Chloride (mg/l)
Lengpui	7.3±0.2	93.5±11.5	149.8±3.3	27.1±1.1	5.49±0.07	362.8±20.1	1.3±0.1	6.6±0.4
Dinthar	7.2±0.1	98.0±11.21	176.1±5.2	24.4±1.0	5.42±0.4	199.3±5.7	0.4±0.01	15.6±1.6
Mission-veng	7.0±0.3	48.8±2.7	135.5±12.0	24.7±0.7	7.5±0.5	117.0±19.9	2.5±0.4	3.35±0.5
Ramrikawn	7.1±0.1	52.3±4.5	100±5.0	27±1.0	3.6±0.08	561.0±25.6	2.1±0.2	26.9±1.3
Sihhmui	6.8±0.06	59.4±6.25	101.5±15.9	28.2±0.4	5.5±0.7	126.0±13.5	0.98±0.02	5.7±0.5

TDS, temperature, alkalinity, pH and hardness.

An. barbirostris was found in most the habitat types, namely stream margin, stream pool, ground pool, ditch, swamp, rice paddy, rock pool, and fish pond habitats and positively associated with pH ($P<0.05$). High alkalinity in the habitat was positively associated with abundance of *An. vagus* ($p<0.05$) while there was a negative association against dissolved oxygen ($p<0.01$). Habitats with alkaline water bodies and slightly turbid with amount of detritus have a positive association against *Cx. quinquefasciatus* ($P<0.05$), found in habitats such as ditches, seapage pools, swallow pits and intradomestic containers where there was huge amount of debris and total dissolved solids but *An. philipinensis* larval abundance was negatively associated with temperature ($P<0.05$) and found mostly in small, open, clear water habitats moreover, positively associated with hardness of water and dissolved oxygen ($P<0.05$). There was a positively associated between *Cx. tritaeniorhynchus* and alkalinity of water ($P<0.05$). The other environmental variables were excluded in the model because they had weaker associations with mosquito larval abundance.

TABLE 4 : Linear regression analysis for the abundance mosquito in Aizawl (showing best water quality predictors)

Species	parameter	Co-efficient	P-value
An. barbirostris	pH	0.30	0.018
An. vagus	DO	-0.29	0.038
	Alkalinity	0.07	0.010
Cx. quinquefasciatus	TDS	0.002	0.022
	pH	0.28	0.017
	Alkalinity	0.01	0.015
An. philipinensis	Hardness	1.86	0.012
	DO	0.63	0.014
	Temperature	-0.44	0.033
Cx. tritaeniorhynchus	Alkalinity	0.08	0.049

DISCUSSION

Understanding the abundance and diversity of mosquito species in the area provides an opportunity to better understand the dynamics of vector borne diseases transmission in different ecosystems[28]. Abundance and distribution of mosquito in the present study showed that *An.* (9 species) was most dominant genus followed by *Culex* (5 species) which was a similar to the survey done by Nagpal and Sharma in1987. Mosquito faunal survey done by Nagpal and Sharma and Malhotra reported the prevalence of *Mansonia*, *Malaya* and *Armigeres* while these mosquitoes were not found during the survey period (2010-2013) but the present study reported the prevalence of *Culiseta* (*Cu.melanura* and *Cu.* inornata) which was the first report so far.

Both quantitative and qualitative characters of the mosquito breeding habitats have contributed to understanding the similarity of habitat requirements of different[14]. Almiron and Brewer[4] pointed out that, different types of habitats, both natural and artificial, nature of vegetation, water movement and water depth were the main characters that explain the observed variations among mosquito species. Cluster analysis done by Almiron and Brewer[4] based on habitat similarity reported that four groups of species have been associated which is similar to the present study that 4 groups are recorded. The phenogram proposed by Almiron and Brewer[4] with 19 operative taxonomic units, is similar with that found in the present study that has 19 operative taxonomic units but different from the phenogram proposed by Devi and Jauhari which has 23 operative taxonomic units.

There are a number of papers on the relationship between vegetation and immature stages by several authors[9-10] and almost all of them reported that larval abundance is related to the presence of a particular kind of vegetation. Their results get support from Adityaa *et al*.[1] who found cemented temporary pools containing maximum food resources, in term of detritus, vegetation and algae allowing the maximum number of species of different guilds to coexist but in the present study, most of the immature mosquitoes were collected from ponds, ditches and river beds. In the present study, immature mosquitos found in turbid water were almost always Culicines, which is similar to the findings of Sattler *et al*.[29]. The preference of Anophelines immatures to breed in clear to slightly turbid water is similar to the findings of Bates and Robert *et al*.. However, Gimning *et al*. found *An. gambiae* larval densities with turbid water bodies. Further, the results of the present findings are contrast to those of Minakawa *et al*. and Edillo *et al*. in having different mosquito species as well as fluctuating ecological conditions prevailing in the area.

Considering the results of the present study in comparison to earlier findings, it has been found that positive associations between mosquito species may result from a common preference for a particular habitat. Maximum immature associations, as recorded in the habitats such as ponds, ditches and river beds, suggest high survival rate, ovipositional preferences and favorable physicochemical characteristics of these habitats. It was also noticed that prolonged water logging with fast changing ecological conditions and extensive surface area of habitats offered favorable breeding conditions to a number of mosquito species including disease vectors. The coexistence of more than one species in a habitat at a given time indicated that mosquito species of the same nature and preference interact with each other[14]. Thus, Understanding mosquito habitats ecology is, therefore, important in designing vector borne diseases control programme[1].

Seasonal variation of mosquito species found in the study was due monsoon that influences temperature, humidity and temporary breeding habitats, which is essential for mosquito survival[14]. Apart from these, rainfall not only provides the medium for the aquatic stages of the mosquito's life but also increases the relative humidity and thereby increasing the longevity of the adult mosquitoes[18]. Therefore, the abundance was increasing during pre-monsoon (Apr-Jun) and the peak was found during monsoon season (Jul-Sep) and gradually declined at post-monsoon.

Mosquitoes use chemical and biological cues to detect the water quality for ovipositing in habitats. Several studies have examined the influence of dissolved oxygen concentration on the abundance of *Anopheles* spp and *Culex* spp with contradicting results[16]. Grillet reported a positive association between dissolved oxygen and the abundance of *An. oswaldoi.* Sunish *et al*. suggested that high algal productivity and associated photosynthesis is responsible for high dissolved oxygen concentrations in aquatic habitats, thereby favoring higher survival of mosquito larvae. In the current study, dissolved oxygen was a best significant factor in productivity *of An. philipinensis and An. vagus* in habitats. Total dissolved solids (TDS), which is the sum of all dissolved organic, inorganic, and suspended solids in water was also a significant factor in larval abundance of *Cx. quinquefasciatus* ($p<0.012$). In most areas of its distribution, *Cx. quinquefasciatus* prefer habitats rich in dissolved matter and such habitats tend to have high TDS[17]. *Cx. tritaerhynchus and Cx. quinquefasciatus* which were mainly collected from ponds containing alkaline water bodies, which shows they can tolerated alkaline habitats. The breeding of *An.* were small, open and clear habitats characterized by high dissolved oxygen, low nutrient levels and normally good quality water[29] and this confirmed the assertion that, *An. philipinensis* had a significant productivity of hardness ($P<0.05$) and dissolved oxygen ($P<0.05$). Besides in small and open habitats, larval predation was less prevalent in temporary habitats than in large permanent habitats[3] and finally, open habitats that tend to produce more algae (the main food source for Anopheline spp).

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